Investigations into the Biosynthesis of Carbocyclic Nucleosides by *Streptomyces citricolor*

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Finally, I would like to express my heartfelt thanks to my parents and last but definitely not least, Ian – thank you.

Contents

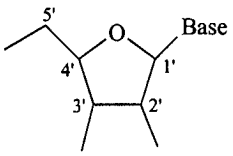
Abbreviations

Ac	acetyl
ADP	adenosine diphosphate
AIBN	azoisobutyronitrile
AMP	adenosine monophosphate
APCI	atmospheric pressure chemical ionisation
ATP	adenosine triphosphate
Bn	benzyl
Boc	*tert*-butoxycarbonyl
bp	boiling point
br	broad
Bu	butyl
Bz	benzoyl
CAL-B	*Candida antarctica* lipase B
d	doublet
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DHP	3,4-dihydro-2*H*-pyran
DIBAL-H	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
EE	ethoxyethyl
e.e.	enantiomeric excess
EI	electron impact
ES	electrospray
Et	ethyl
FAB	fast atom bombardment
HMDS	hexamethyldisilazane
HPLC	high performance liquid chromatography
IBX	*o*-iodoxybenzoic acid
LDA	lithium diisopropylamide
m	multiplet
MCPBA	*m*-chloroperbenzoic acid
Me	methyl
MOM	methoxymethyl
mp	melting point
Ms	methylsulfonyl (mesyl)
NBS	*N*-bromosuccinimide
NMO	4-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance

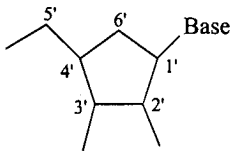
nOe	nuclear Overhauser enhancement
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
PG	protecting group
Ph	phenyl
PMB	*p*-methoxybenzyl
PPTS	pyridinium *p*-toluenesulfonate
Pr	propyl
PRPP	5-phosphoribosyl-α-1-pyrophosphate
PTSA	*p*-toluenesulfonic acid
Red-Al	sodium bis(2-methoxyethoxy)aluminium hydride
s	singlet
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
Tf	trifluoromethylsulfonyl (triflyl)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPDS	tetraisopropyldisilane
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
Ts	*p*-tolylsulfonyl (tosyl)
Z	benzyloxycarbonyl

Numbering System

The numbering system of the carbocyclic nucleoside analogues and their precursors agrees with the numbering employed for nucleosides with the carbon replacing the oxygen of the furanose ring being designated as C-6'.

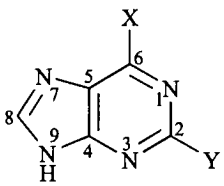


Nucleoside



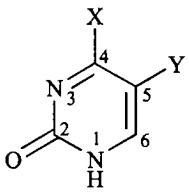
Carbocyclic nucleoside

Bases:



adenine X = NH_2, Y = H
guanine X = OH, Y = NH_2
hypoxanthine X = OH, Y = H

Purines



cytosine X = NH_2, Y = H
thymine X = OH, Y = CH_3
uracil X = OH, Y = H

Pyrimidines

Abstract

Aristeromycin **4** and neplanocin A **5** are biologically active carbocyclic nucleosides produced by the organism *Streptomyces citricolor*. Previous studies towards elucidating their biosynthesis have led to a proposed biosynthetic pathway in which D-glucose is converted *via* a number of carbocyclic intermediates to neplanocin A and aristeromycin (Figure 1.2).

This thesis describes the studies that have been carried out in order to:

i. identify the first formed carbocyclic intermediate on the biosynthetic pathway (Chapter 2).
ii. determine the identity of the phosphorylated intermediates prior to neplanocin A (Chapter 3).

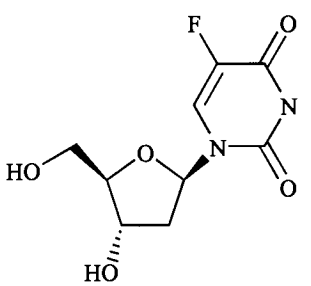
The novel (2*R*,3*S*,4*R*), (2*S*,3*R*,4*S*) and (2*R*,3*S*,4*S*) keto-tetrols **95**, ***ent*-95** and **116** have been prepared and their syntheses are described. To determine whether these intermediates lie on the biosynthetic pathway, feeding studies have been carried out using a mutant of *Streptomyces citricolor*. The results of these studies, described within, suggest that both the (2*R*,3*S*,4*R*) keto-tetrol **95** and its diastereomer (2*R*,3*S*,4*S*) keto-tetrol **116** lie on the biosynthetic pathway.

The syntheses of the known intermediate tetrol **83a**, the proposed phosphorylated intermediate 5-phosphate **83b** and the corresponding 1-phosphate **152**, *via* multi-step syntheses from the cyclopentenone **61**, prepared from either D-ribose **126** or cyclopentadiene **36**, are described.

1 Introduction

1.1 Nucleoside Analogues as Therapeutic Agents

Nucleosides and their analogues display a wide range of biological activities and have attracted particular attention as anti-tumour and anti-viral agents. For example, 5-fluoro-2'-deoxyuridine **1** shows anti-cancer activity; 2'-fluoro-5-iodo-1-β-D-arabinofuranosylcytosine (FIAC) **2** displays anti-herpes simplex virus properties; and 3'-azido-3'-deoxythymidine (AZT) **3** has been used for the treatment of the human immunodeficiency virus (HIV) infection.[1]



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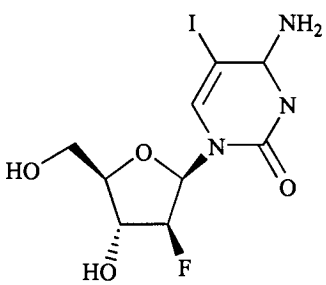

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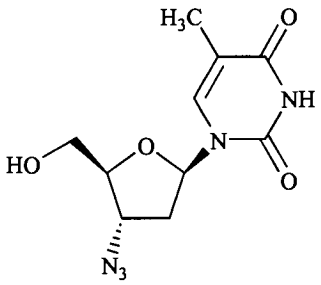

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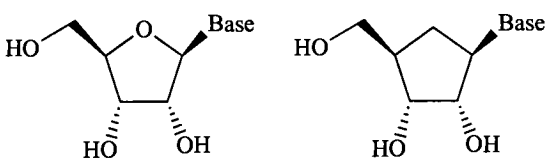
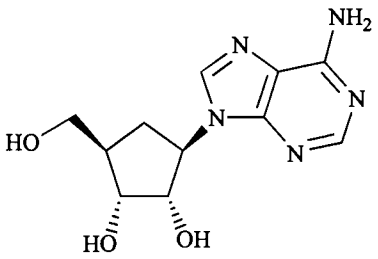
As a result of their properties many modifications have been made to both the heterocyclic base and the sugar moiety. One such modification is the replacement of the oxygen atom of the furanose ring by a methylene group as shown in Figure 1.1. This change is of particular interest since the resulting carbocyclic nucleosides exhibit biological activity as well as possessing greater metabolic stability to the phosphorylase enzymes, which cleave the N-glycosidic linkage between the heterocyclic base and the sugar of normal nucleosides.



nucleoside carbocyclic nucleoside

Figure 1.1 Comparison of a nucleoside and a carbocyclic nucleoside

Interest in the synthesis and biological activities of carbocyclic nucleoside analogues was stimulated by the discovery that the naturally occurring carbocyclic nucleosides aristeromycin **4** and neplanocin A **5** displayed potent biological activity.[2,3] Aristeromycin and neplanocin A will be discussed in more detail in Section 1.3.



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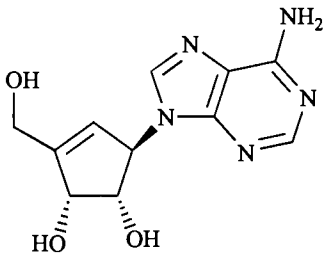

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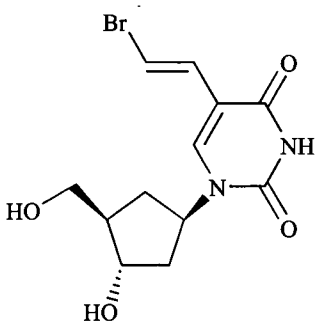
For the past fifteen years much of the research on the chemistry of nucleoside analogues has been directed towards the development of agents showing activity against HIV. More effective treatment has also been sought for other viral infections, in particular herpes simplex virus (HSV types 1 and 2), varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV) and hepatitis B virus (HBV), since infections of these types can be potentially lethal to AIDS patients and other immunocompromised individuals. The search for clinically useful nucleoside analogues has resulted in an explosion of synthetic activity in the field of carbocyclic nucleosides which has led to the discovery of several derivatives with potent anti-viral activity. For example, carbocyclic 5-bromovinyl-deoxyuridine (BVDU) **6** which has activity against HSV and VZV infections and carbocyclic 2'-*ara*-fluoroguanosine **7** which is more active against HSV-1 and HSV-2 than its natural nucleoside analogue.[1,4-7]



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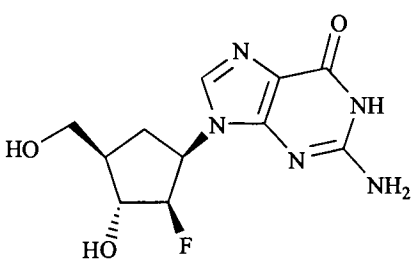

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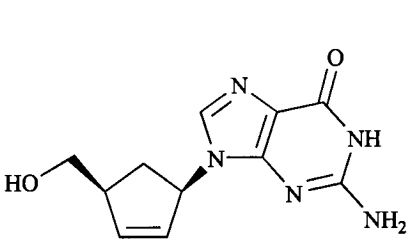
Presently, another important area in which carbocyclic nucleosides are receiving attention is as building blocks for antisense oligonucleotides.[8]

Of particular interest amongst the carbocyclic nucleosides are carbovir **8** and its 6-cyclopropylaminopurine analogue 1592U89 (abacavir) **9**, as both compounds possess potent anti-HIV activity.[9,10] Carbovir **8** is in clinical use for the treatment of the acquired immunodeficiency syndrome (AIDS) but its long-term usefulness is limited because of its toxicity. However, abacavir **9** operates by a unique pathway which enables it to overcome the toxicological deficiencies of carbovir **8** whilst maintaining potent and selective anti-HIV activity.[11] Abacavir **9** has completed all clinical trials and is being marketed by GlaxoWellcome under the name of Ziagen®.



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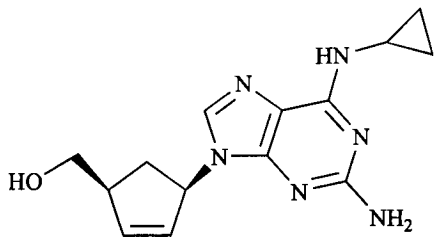

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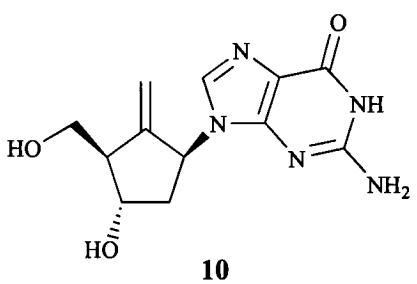

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The exact mechanism of action of these compounds is not completely known, however it is clear that they are pro-drugs and are sequentially phosphorylated by cellular kinases to the corresponding triphosphates. The triphosphate is then incorporated into the replicating viral DNA chain by HIV reverse transcriptase, and chain termination results as there is no 3'-hydroxyl group for further elongation of the chain. In addition, the 5'-triphosphate of the oligonucleotide may act as a competitive inhibitor of the reverse transcriptase.[6]

A further carbocyclic nucleoside of interest is BMS-200475 **10**, which contains an *exo*-cyclic double bond. This compound displays potent anti-hepatitis B virus activity and is currently in phase II clinical trials at Bristol-Myers-Squibb.[12]

1.2 The Synthesis of Chiral Carbocyclic Nucleosides

The pharmaceutical importance of carbocyclic nucleoside analogues has focused attention on new syntheses of these compounds. Furthermore, since the biological activity of a nucleoside analogue normally resides in one enantiomer, and with the increasing demand for new drug substances to be enantiomerically pure, the development of enantioselective routes to carbocyclic nucleosides is of paramount importance. In this thesis the literature from the beginning of 1997 to the end of 1999 is discussed. The reader is referred to earlier reviews for coverage of the literature prior to 1997.[1,4-6]

1.2.1 Methods for coupling the heterocyclic base with the carbocyclic ring

Carbocyclic nucleosides are generally prepared by initial formation of a functionalised cyclopentane or cyclopentene ring followed by coupling of a purine or pyrimidine base. The heterocyclic base can either be incorporated intact onto the carbocycle in a convergent approach, or constructed around an amino function on the carbocycle in a linear synthesis.

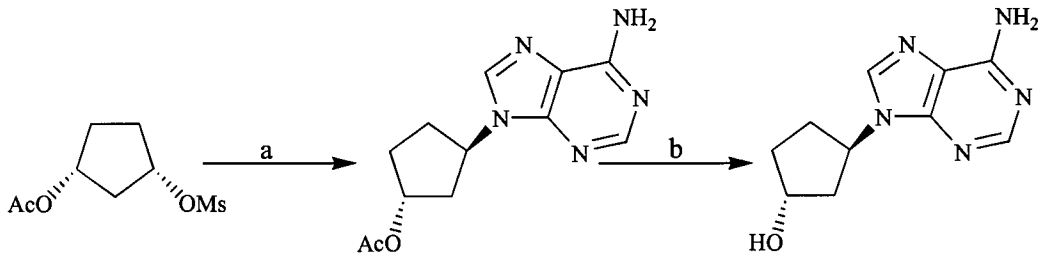
(a) Convergent approaches

Direct coupling of the heterocyclic base to a functionalised carbocyclic ring is the most common approach for the synthesis of carbocyclic nucleosides. This route does however introduce the problem of regioselectivity with respect to attack by the heterocyclic base. In the case of purines, attachment at the N9, N7 and N3 nitrogen atoms is possible and all three regioisomers are often observed.

Five main ways in which the base can be directly coupled to the carbocyclic ring have been described:

(i) Nucleophilic substitution of an activated hydroxyl group

The displacement of a mesylate, tosylate, triflate or halide can be used for the direct coupling as illustrated in the preparation of MDL 201449A **11** (Scheme 1.1).[13] This reaction takes place with a net inversion of the hydroxyl stereochemistry.



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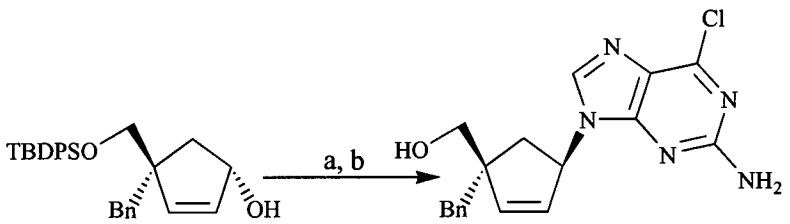
Scheme 1.1 *Reagents and conditions:* a. adenine, NaH, DMF, 55-60°C, 53%; b. 6 M HCl, EtOH, reflux, 87%.

(ii) Mitsunobu reaction

One of the most common methods used is the Mitsunobu reaction (Scheme 1.2).[14]



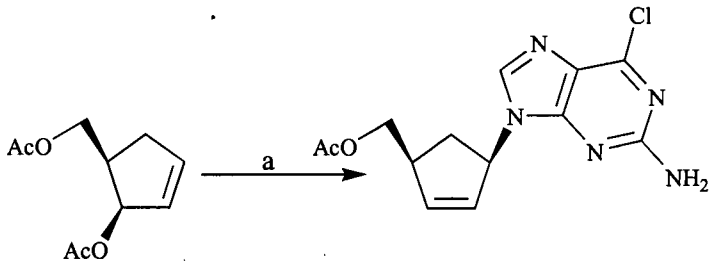
Scheme 1.2 *Reagents and conditions:* a. 2-amino-6-chloropurine, $EtO_2CN{=}NCO_2Et$, PPh_3; b. TBAF, 36%.

Activation of the secondary alcohol by a complex formed from an azodicarboxylate and triphenylphosphine allows direct substitution with net inversion of stereochemistry.

A disadvantage of this approach is the laborious purification procedures which are often associated with Mitsunobu reactions for the removal of triphenylphosphine oxide from the final product.[15]

(iii) Palladium catalysed displacement of an allylic ester or carbonate

The most recently described and extremely useful strategy for the coupling is the palladium(0) catalysed substitution of allylic esters, carbonates or epoxides (Scheme 1.3).[16] A variety of Pd(0) catalysts have been employed.

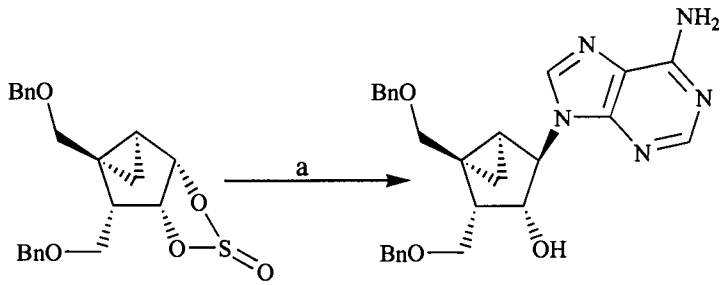


Scheme 1.3 *Reagents and conditions:* a. 2-amino-6-chloropurine, $Pd(PPh_3)_4$, NaH, THF, DMSO, 65%.

This reaction which was initially described by Trost[17] proceeds with retention of configuration, although allylic rearrangement can occur.

(iv) Ring opening of an epoxide

Epoxides, cyclic sulfates and sulfites can be used for the coupling of the base to the carbocyclic ring (Scheme 1.4).[18]

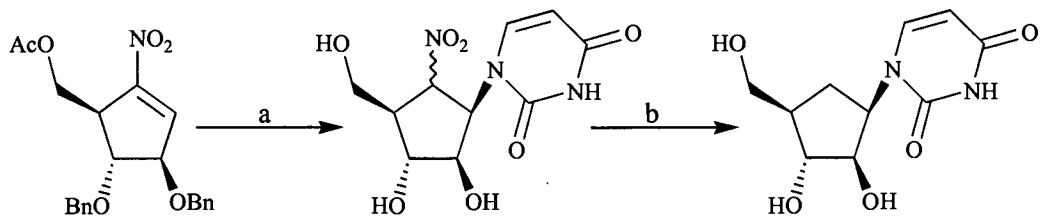


Scheme 1.4 *Reagents and conditions:* a. adenine, NaH, DMF, 18-crown-6, 50%.

A limit of this method is the lack of regioselectivity in the opening of the epoxide, cyclic sulfate and sulfite. Nucleophilic attack can occur at two sites which leads to a mixture of isomers being formed.

(v) Michael 1,2 addition

A small number of examples which involve the Michael addition of a heterocyclic base to a nitrocyclopentene derivative have been described (Scheme 1.5).[19]



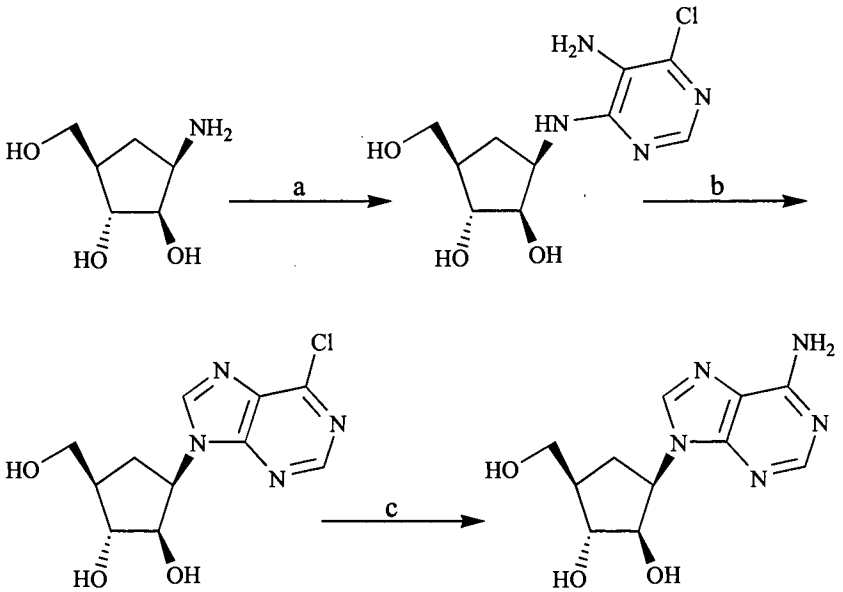
Scheme 1.5 *Reagents and conditions:* a. CsF, silylated uracil, DMF, 50%; b. Bu_3SnH, AIBN, 21%.

(b) Linear approaches

In a linear approach carbocyclic nucleosides are prepared by construction of the purine or pyrimidine base from the appropriate precursor onto a cyclopentylamine. The use of a linear synthesis is less common due to the increased number of steps which results in low overall yields, however it does have the advantage that a large number of carbocyclic nucleoside analogues with modified bases can be easily prepared and then screened for biological activity.

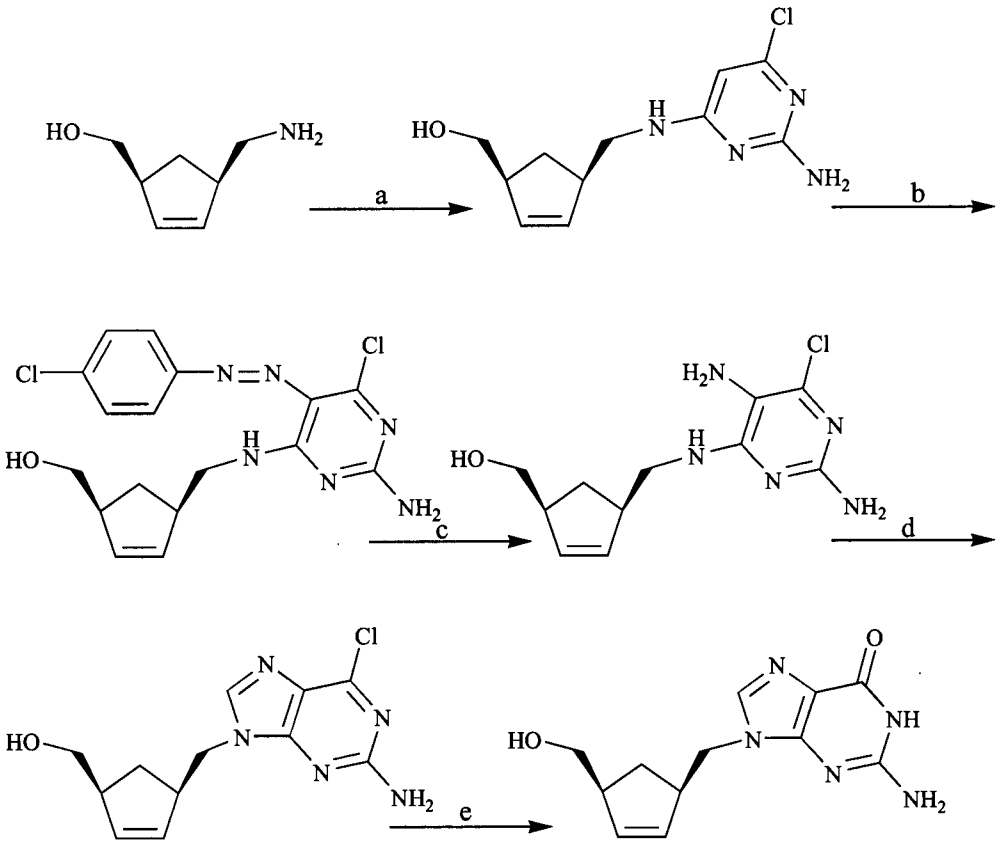
(i) Synthesis of purines

The synthesis of adenine derivatives is carried out as shown in Scheme 1.6.[20]



Scheme 1.6 *Reagents and conditions:* a. 5-amino-4,6-dichloropyrimidine, Et_3N, *t*-BuOH, reflux, 78%; b. $CH(OEt)_3$, 12 M HCl, 70%; c. NH_3, dioxane, 80°C, 100%.

Guanosine derivatives can be synthesised by a similar procedure which is outlined in Scheme 1.7.[21]

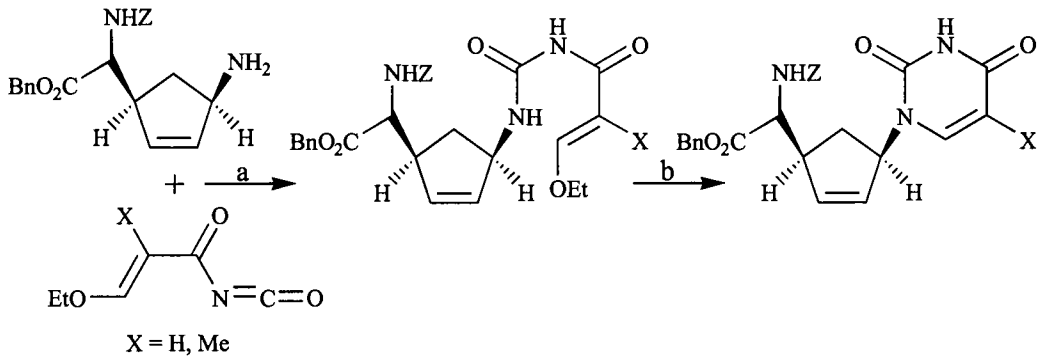


Scheme 1.7 *Reagents and conditions:* a. 2-amino-4,6-dichloropyrimidine, *i*-Pr_2NEt, BuOH, reflux, 80%; b. 4-$ClC_6H_4N_2^+Cl^-$, AcOH, NaOAc, H_2O, 69%; c. Zn, AcOH, EtOH, H_2O, 50%; d. $CH(OEt)_3$, HCl, H_2O; e. NaOH, H_2O, 74%.

Other nucleophiles such as amines and alkoxides can be used to incorporate a variety of substituents at the 6-position of guanine or adenine.

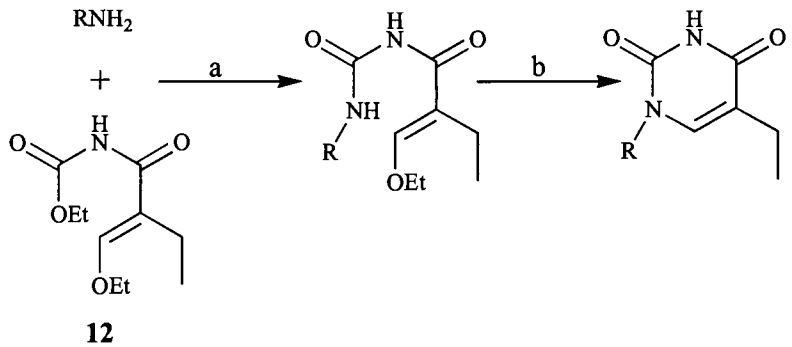
(ii) Synthesis of pyrimidines

Uridine and thymidine derivatives can be synthesised by a procedure originally developed by Shaw and Warrener[22,23] which is shown in Scheme 1.8.[24]



Scheme 1.8 *Reagents and conditions:* a. benzene-DMF, 85%; b. 1 M H_2SO_4, dioxane, reflux, 88%.

These pyrimidine derivatives can also be prepared by the reaction of the carbocyclic amine with 3-ethoxy-N-carboethoxy-2-ethylacrylamide **12** as described in Scheme 1.9.[6]



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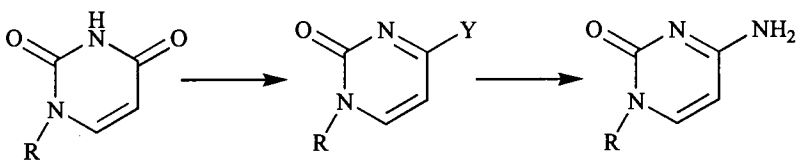
Scheme 1.9 *Reagents and conditions:* a. Et_3N, dioxane; b. 2 M HCl, dioxane, 80% 2 steps.

Various substituents can be incorporated at the 5-position of the pyrimidine by this approach.

Cytidine derivatives can be prepared from the uridine analogue **13** by ammonolysis of either the 4-chloro **14**, 4-methylthio **15** or 4-(1,2,4-triazol-1-yl) **16** derivatives (Scheme 1.10).[5]



13

14 Y = Cl
15 Y = MeS
16 Y = 1,2,4-triazol-1-yl

Scheme 1.10

1.2.2 Syntheses of chiral carbocyclic rings

Substantial efforts have been made towards the enantioselective synthesis of the carbocyclic ring moiety of carbocyclic nucleosides. Two main approaches have been described:

i. Synthesis from racemic/achiral compounds such as norbornene and cyclopentadiene, which require enzymatic or chemical resolution at some point in the synthesis in order to obtain chiral compounds.
ii. Synthesis using a starting material from the chiral pool *e.g.* a carbohydrate.

More recently, the use of asymmetric synthetic methods for the enantioselective synthesis of carbocyclic nucleosides have been described.[25-32] Methods such as asymmetric cycloadditions, catalytic asymmetric desymmetrisations and chiral auxiliary-based asymmetric reactions have been employed.

In this thesis, only the approaches that exploit synthesis by enzymatic resolution or from the chiral pool will be discussed.

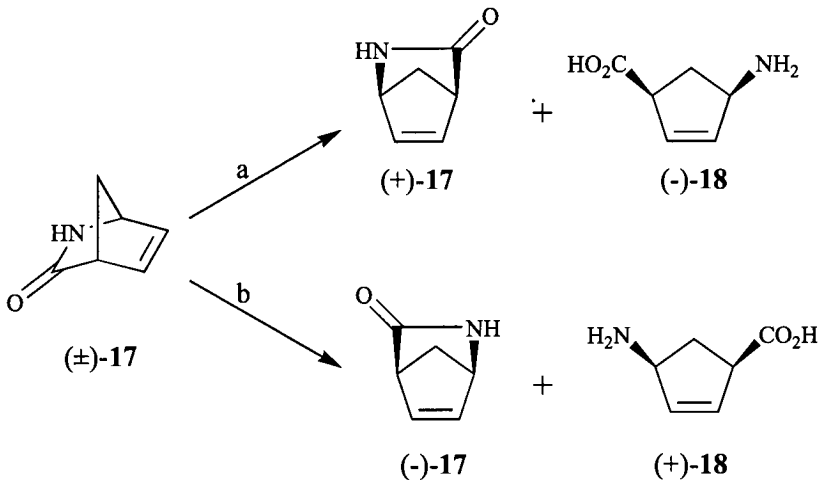
(a) Enantioselective synthesis by enzymatic resolution

The application of enzymes to organic synthesis is one of the most useful and practical methods for the preparation of chiral compounds in high optical purity. The enzymatic desymmetrisation of *meso* intermediates and enzymatic resolution of chiral, racemic mixtures have commonly been used for the enantioselective synthesis of carbocyclic nucleosides. Desymmetrisation of *meso* intermediates offers the advantage that all the material can be utilised.

(i) Synthesis from bicyclic lactams

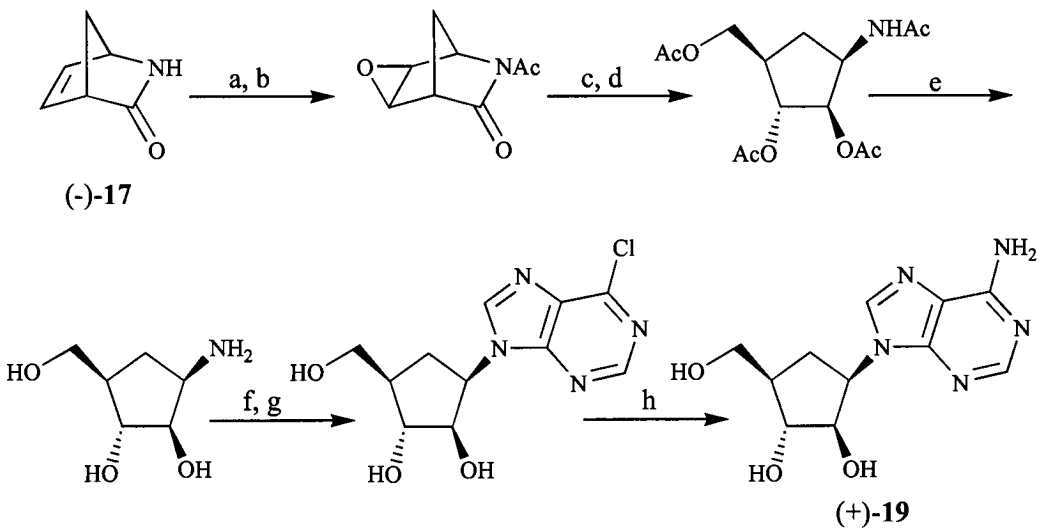
The bicyclic lactam, 2-azabicyclo[2.2.1]hept-5-en-3-one **17**, which is derived from the cycloaddition product of cyclopentadiene and tosyl cyanide by aqueous hydrolysis,[33,34] is a versatile building block in the synthesis of many carbocyclic nucleosides. Both optically pure forms of the lactam (+)- and (-)-**17** are

commercially available and are obtained *via* enzymatic resolution of the racemic azanorbornenone (±)-**17** using *Pseudomonas fluorescens* (ENZA22) or *Aureobacterium* (ENZA25) (Scheme 1.11).[35] The amino acids (+)- and (-)-**18** which are produced from this enzymatic hydrolysis with high optical purity are also useful intermediates for the synthesis of carbocyclic nucleosides.[9]



Scheme 1.11 *Reagents and conditions:* a. *Aureobacterium* (ENZA25); b. *Pseudomonas fluorescens* (ENZA22).

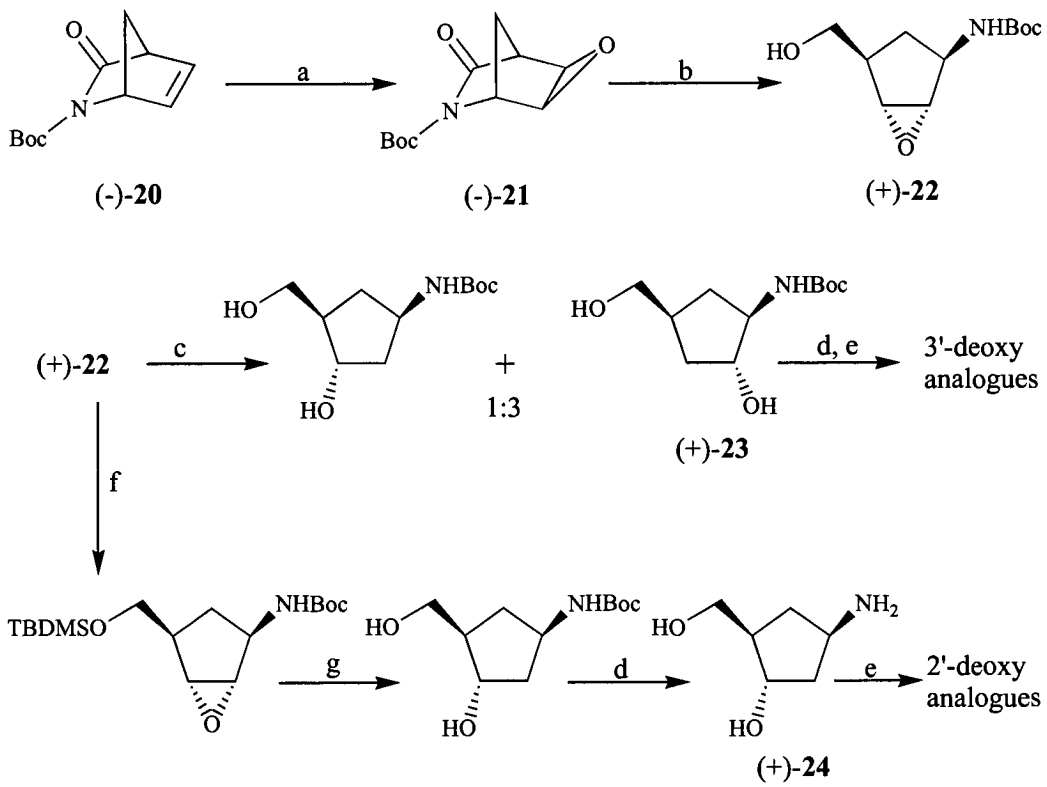
Katagiri *et al.*[20] have developed an efficient synthesis of carbocyclic cyclaridine (+)-**19**, an adenosine deaminase-resistant *ara*-A derivative, from the laevorotatory lactam (-)-**17** as shown in Scheme 1.12.



Scheme 1.12 *Reagents and conditions:* a. Ac_2O, Et_3N, DMAP, $CHCl_3$, 78%; b. MCPBA, $CHCl_3$, 68%; c. $NaBH_4$, MeOH; d. Ac_2O, pyridine, 63% 2 steps; e. 2 M HCl, 70°C; f. 5-amino-4,6-dichloropyrimidine, Et_3N, *t*-BuOH, reflux, 78% 2 steps; g. $CH(OEt)_3$, 12 M HCl, 70%; h. NH_3, dioxane, 80°C, 100%.

This procedure involves the regioselective cleavage of an epoxide ring by neighbouring participation of an N-acyl amino group.

Elaboration of the bicyclic lactam epoxide into intermediates suitable for the synthesis of 2'-deoxy- and 3'-deoxy-cyclopentyl carbocyclic nucleosides has recently been reported by Cullis (Scheme 1.13).[36]

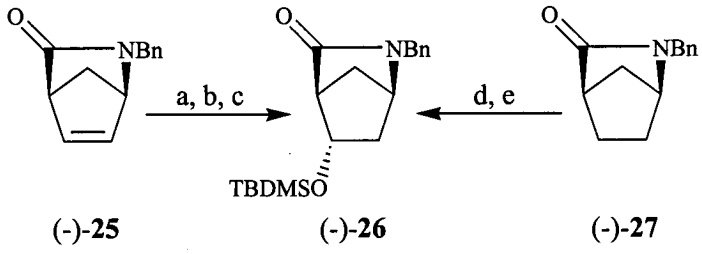


Scheme 1.13 *Reagents and conditions:* a. MCPBA, CH_2Cl_2, 71%; b. $NaBH_4$, MeOH, 89%; c. Red-Al, toluene, 71%; d. H_2O, reflux, 100%; e. standard base synthesis; f. TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 97%; g. Red-Al, toluene, 85%.

The N-Boc bicyclic lactam **20**, prepared by treatment of bicyclic lactam (-)-**17** with Boc anhydride, was reacted with MCPBA to give the *exo* epoxide **21**. Reduction of **21** with sodium borohydride afforded the cyclopentyl epoxide **22**, which was converted to cyclopentylamines **23** and **24**. These intermediates provide a convenient route to 2'-deoxy- and 3'-deoxy- carbocyclic nucleoside analogues.[36]

Carbocyclic 2'-deoxy-nucleosides have also been prepared from the 5-*exo* hydroxy lactam **26**.[37] Compound **26** has been synthesised by Palmer *et al.*[37,38] using either a microbial biohydroxylation of the saturated lactam **27**, prepared by

hydrogenation of the bicyclic lactam (-)-**17**; or an oxymercuration reaction of the unsaturated N-benzyl lactam **25** (Scheme 1.14).

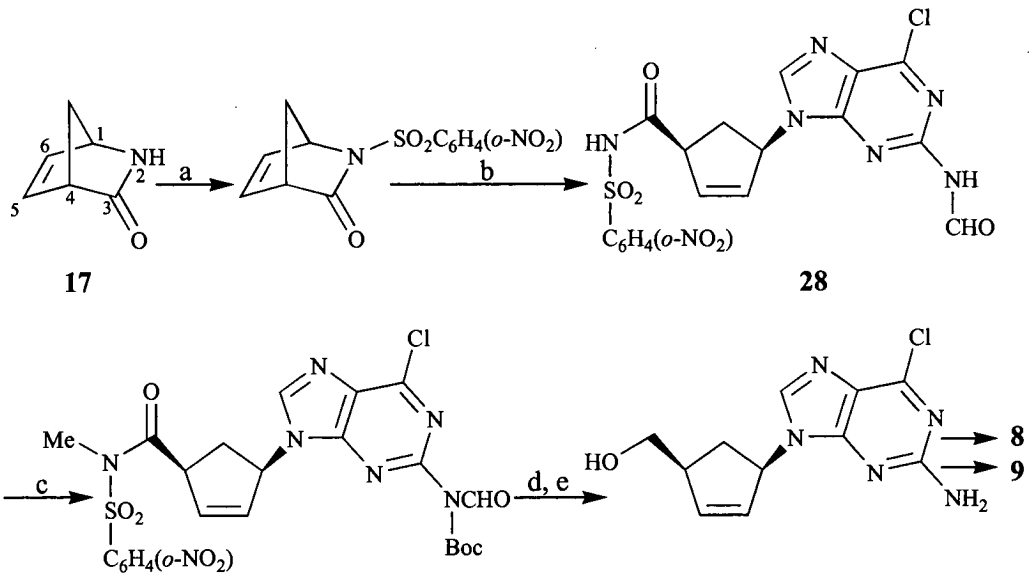


(-)-**25** (-)-**26** (-)-**27**

Scheme 1.14 *Reagents and conditions:* a. $Hg(OAc)_2$, aq. THF; b. $NaBH_4$; c. TBDMSCl, imidazole; d. *Beauveria bassiana*; e. TBDMSOTf, 2,6-lutidine.

This biohydroxylation has the potential to be developed into a synthetically useful procedure for the preparation of multigram quantities of the useful protected alcohol **26**.[37]

All previous syntheses of carbocyclic nucleosides from the bicyclic lactam **17** involve cyclopentylamines as intermediates, which are formed by 2,3-bond fission of the lactam **17**. These methods have the disadvantage that the time-consuming reaction steps associated with purine ring construction result in low overall yields. Katagiri and co-workers[39] were the first to report the efficient synthesis of carbovir **8** and abacavir **9** from lactam **17** *via* π-allylpalladium complex formation by ring strain-assisted C-N (1,2-) bond cleavage (Scheme 1.15).



17 **28**

Scheme 1.15 *Reagents and conditions:* a. i. *n*-BuLi, THF, -78°C; ii. $(o\text{-}NO_2)C_6H_4SO_2Cl$, 83%; b. i. $Pd[P(Oi\text{-}Pr)_3]_4$, THF; ii. 2-(formylamino)-6-chloropurine (Bu_4N^+ salt), 55%; c. i. NaH, THF, 0°C; ii. Boc_2O; iii. MeI; d. $NaBH_4$, MeOH; e. AcOH, 72% 5 steps.

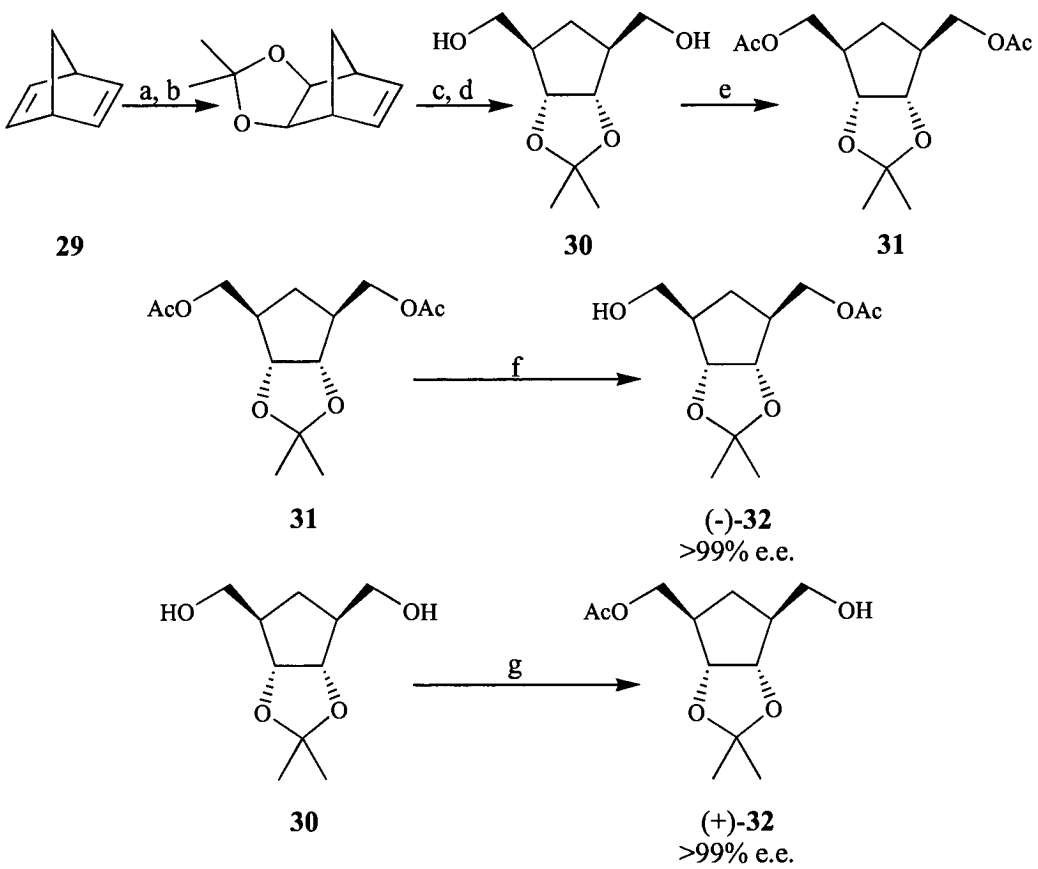
Activation of the nitrogen of lactam **17** with the electron-withdrawing *o*-nitrobenzenesulfonyl group facilitated the palladium catalysed coupling of 2-(formylamino)-6-chloropurine to give sulfonamide **28**.

The enantiomerically pure bicyclic lactam (+)-**17** has also been prepared by asymmetric Diels-Alder cycloaddition using a chiral auxiliary.[40]

(ii) Synthesis from norbornane-type compounds

Several approaches for the synthesis of carbocyclic rings from compounds possessing the norbornane-type framework have been reported.[41-43] The easy availability of these types of compounds in large quantities makes them attractive starting materials.

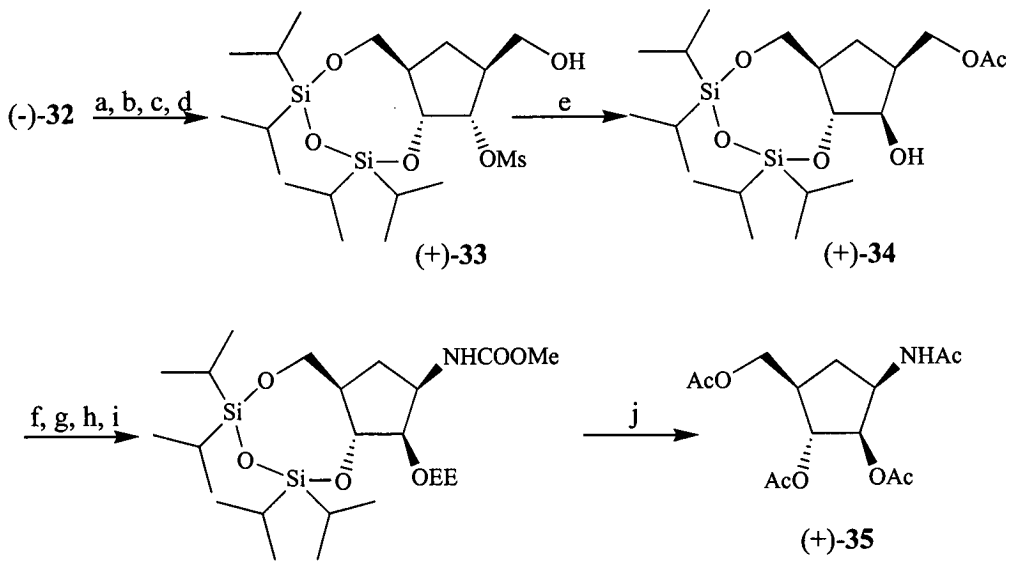
The *meso*-diacetate **31** has been prepared in five steps from norbornadiene **29** as shown in Scheme 1.16.[43]



Scheme 1.16 *Reagents and conditions:* a. OsO_4, NMO; b. acetone-H^+, 46% 2 steps; c. O_3; d. $NaBH_4$, 93% 2 steps; e. Ac_2O, pyridine, 73%; f. *Pseudomonas fluorescens* lipase, phosphate buffer (pH 7.0), 71%; g. *Pseudomonas fluorescens* lipase, vinyl acetate, 81%.

Enzyme-catalysed hydrolysis of the *meso*-diacetate **31** using *Pseudomonas fluorescens* lipase[44] afforded the monoacetate (-)-**32**. The opposite enantiomer (+)-**32** was also prepared by transesterification of the *meso*-diol **30** using *Pseudomonas fluorescens* lipase.[43] This procedure provides a practical route for the synthesis of both enantiomers of carbocyclic nucleosides (Scheme 1.16).

Recently, Suemune *et al.*[45] have used the monoacetate (-)-**32** to synthesise the intermediate (+)-**35** as outlined in Scheme 1.17.



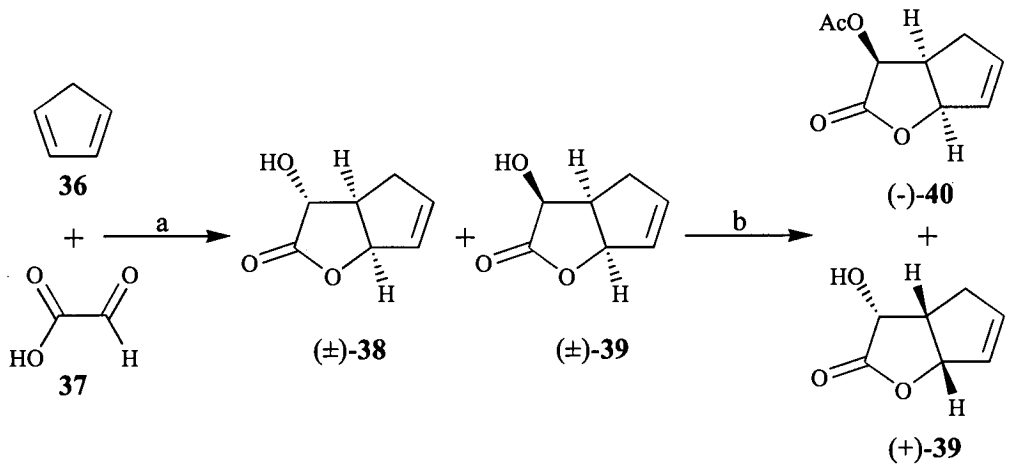
Scheme 1.17 *Reagents and conditions:* a. HCl, THF, 90%; b. $TIPDSCl_2$, pyridine, 96%; c. MsCl, Et_3N, 98%; d. K_2CO_3, MeOH, 72%; e. CsOAc, DMF, 65%; f. CH_2CHOEt, PPTS, 96%; g. K_2CO_3, MeOH, 81%; h. RuO_2, $NaIO_4$, 73%; i. DPPA, Et_3N, MeOH, 40%; j. i. KOH, MeOH; ii. HCl; iii. Ac_2O, pyridine, 20%.

In this synthetic route inversion of the stereochemistry at the C-2 position was achieved by treatment of the mesylate derivative (+)-**33** with cesium acetate with subsequent migration of the acetyl group to the primary alcohol to give compound (+)-**34**.

The cyclopentylamine (+)-**35** was then used to prepare the anti-HSV active carbocyclic analogue of *ara*-A, (+)-cyclaridine **19**, by the stepwise construction of adenine following standard procedures.[20]

(iii) Synthesis from bicyclic lactones

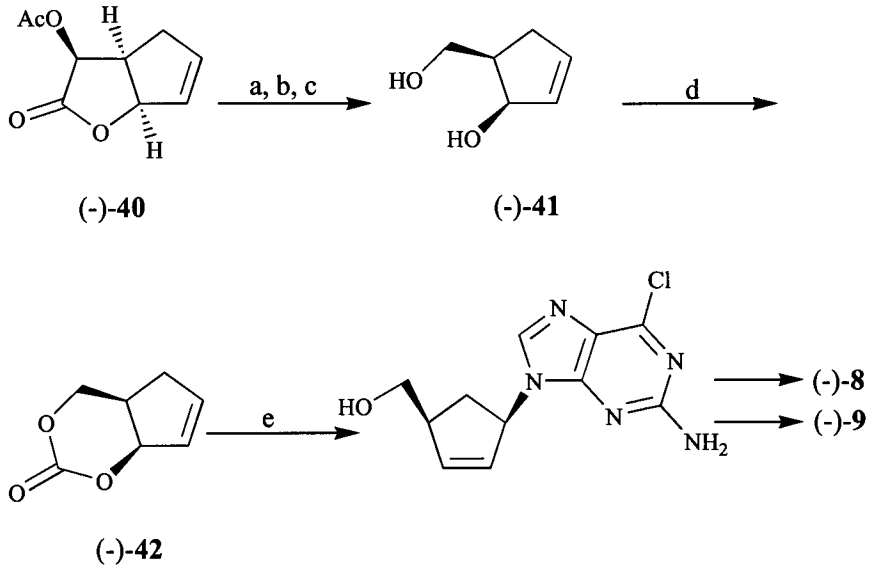
The hydroxylactone, 4-*endo*-hydroxy-2-oxabicyclo[3.3.0]oct-7-en-3-one, **39** is a common starting material for the synthesis of carbocyclic nucleosides as both enantiomers are available in optically pure form by enzymatic resolution. The racemic *endo*-hydroxylactone (±)-**39** is prepared by the water-promoted reaction of glyoxylic acid **37** with cyclopentadiene **36** to give a 1:4 mixture of lactones **38** and **39**, which can be separated by chromatography.[46] The major isomer, (±)-**39** is resolved by enzymatic esterification to yield the optically pure (-)-acetyl ester (-)-**40** and the (+)-hydroxylactone (+)-**39** (Scheme 1.18). Using the same enzyme, the acetyl ester (±)-**40** can be resolved by hydrolysis in phosphate buffer solution to yield the (-)-hydroxylactone (-)-**39** and the (+)-acetyl ester (+)-**40**.[11,47]



Scheme 1.18 *Reagents and conditions:* a. H_2O, 65%; b. *Pseudomonas fluorescens* lipase, vinyl acetate.

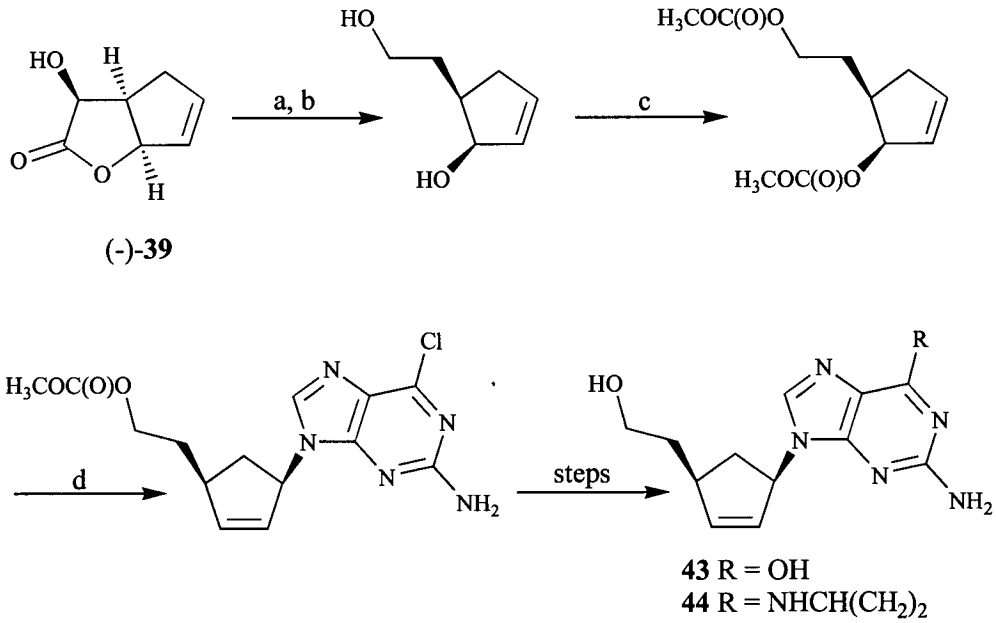
Olivo *et al.*[11] have reported the synthesis of the bicyclic carbonate **42** from the optically pure *endo*-hydroxylactones **39** to provide a new route to both enantiomers of carbovir **8** and 1592U89 **9** (Scheme 1.19). This route avoids the protection-deprotection steps which have been utilised in previous syntheses.[48]

The diol (-)-**41** has also been synthesised asymmetrically by Crimmins, using a combination of an asymmetric aldol condensation with ring closing metathesis.[16]



Scheme 1.19 *Reagents and conditions:* a. $LiAlH_4$, THF, reflux; b. $NaIO_4$, Et_2O-H_2O; c. $NaBH_4$, EtOH, 75% 3 steps; d. triphosgene, Et_3N, CH_2Cl_2, 76%; e. 2-amino-6-chloropurine, $Pd(PPh_3)_4$, DMSO-THF, 53%.

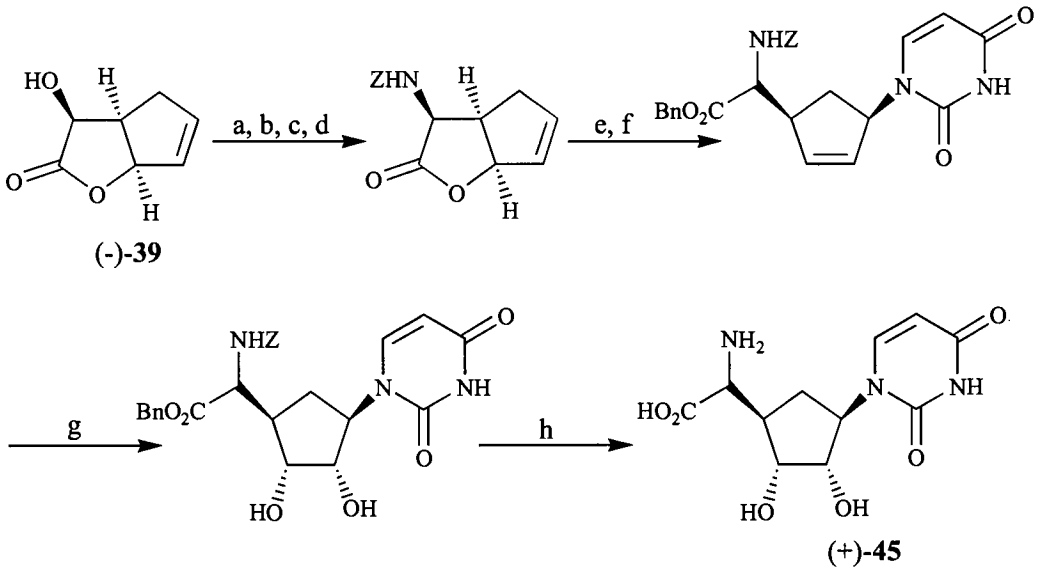
Olivo[49] have also used the enzymatically resolved hydroxylactone (-)-**39** as the starting material in a short, enantioselective synthesis of 5'-*homo*-carbovir **43** and 5'-*homo*-1592U89 **44** as described in Scheme 1.20.



Scheme 1.20 *Reagents and conditions:* a. PPh_3, $ZnBr_2$, DEAD; b. $LiAlH_4$, THF, 90% 2 steps; c. $ClCO_2CH_3$, pyridine, CH_2Cl_2, 70%; d. 2-amino-6-chloropurine, $Pd(PPh_3)_4$, THF-DMSO, 60%.

This practical methodology can also provide the opposite enantiomer 5'-*homo*-carbocyclic nucleosides.[49]

The synthesis of carbocyclic uracil polyoxin C **45**, in which the 5'-position is substituted by an amino acid, from the hydroxylactone (-)-**39** has been accomplished by Aggarwal *et al.*[24] as outlined in Scheme 1.21.

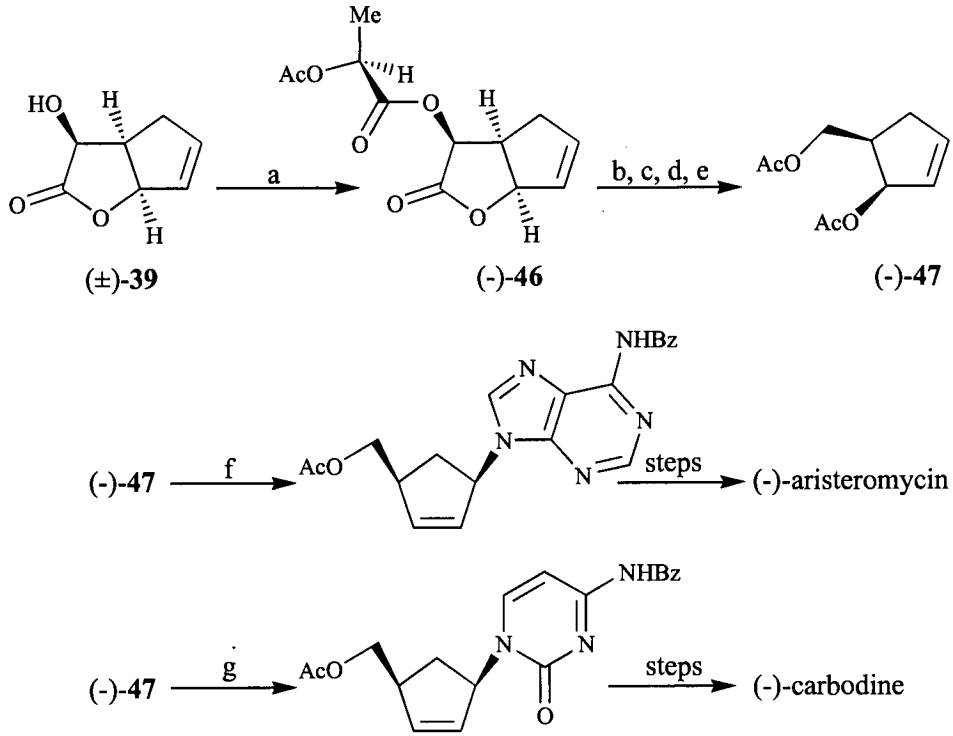


Scheme 1.21 *Reagents and conditions:* a. $ZnBr_2$, PPh_3, DEAD, THF, 60%; b. NaN_3, DMSO, 87%; c. PPh_3, THF-H_2O, 92%; d. ZCl, $NaHCO_3$, THF-H_2O, 0°C, 94%; e. uracil, HMDS, TMSCl, $Pd(PPh_3)_4$, MeCN; f. BnBr, $NaHCO_3$, DMF, 50% 2 steps; g. OsO_4, NMO, THF, 44%; h. H_2, Pd/C, EtOH-H_2O, 100%.

This synthesis involves the regio- and stereoselective palladium-mediated substitution reaction of an α-amino substituted unsaturated lactone with bis-silylated uracil.

The hydroxylactone (±)-**39** has also been resolved chemically by conversion to the 2-acetoxypropionate derivative **46** followed by separation of the diastereomers by chromatography as shown in Scheme 1.22.[6,50]

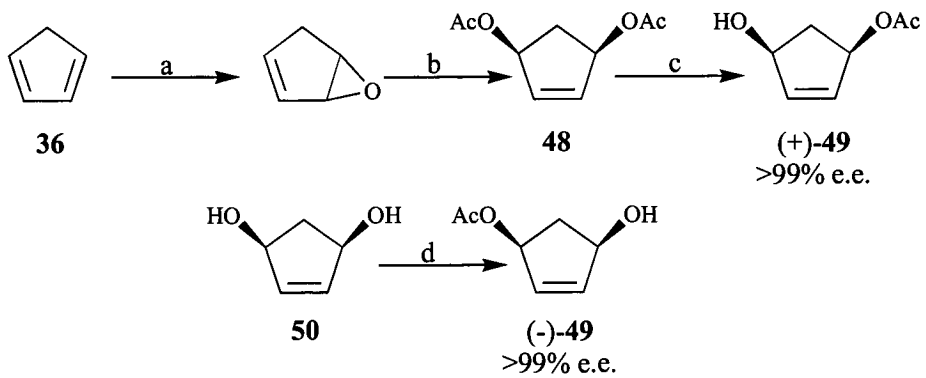
The enantiomerically pure lactone derivative (-)-**46** can then be converted to the carbocyclic nucleosides (-)-aristeromycin and (-)-carbodine *via* the diacetate (-)-**47** (Scheme 1.22).[8]



Scheme 1.22 *Reagents and conditions:* a. (*S*)-O-acetyllactyl chloride, pyridine, 0°C, 91%; b. $LiAlH_4$, THF; c. $NaIO_4$, Et_2O-H_2O; d. $NaBH_4$, MeOH; e. Ac_2O, DMAP, pyridine, 30% 4 steps; f. N6-benzoyladenine (Cs^+ salt), $Pd(PPh_3)_4$, DMF, 55°C, 43%; g. N6-benzoylcytosine (Cs^+ salt), $Pd(PPh_3)_4$, DMF, 55°C, 85%.

(iv) Synthesis via the monoacetate of cyclopent-2-ene-1,4-diol

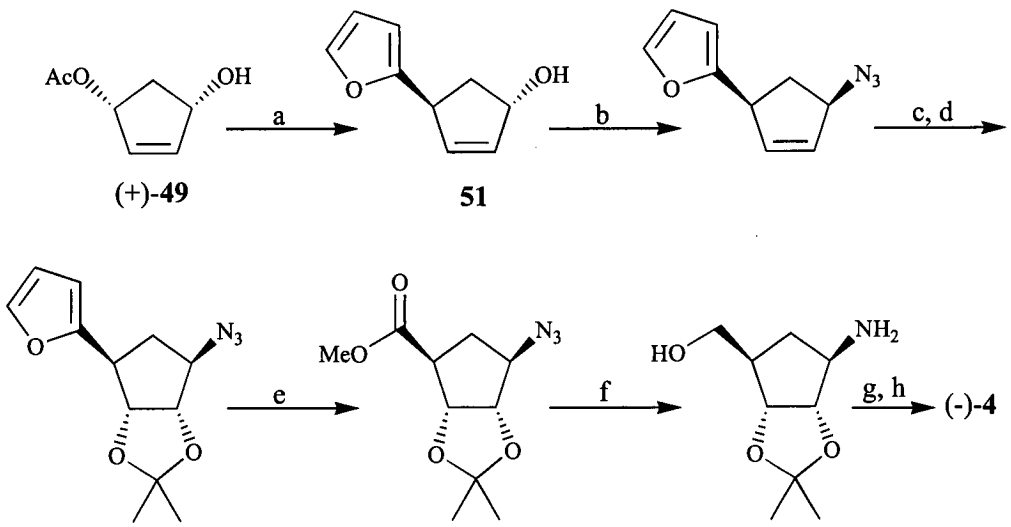
The monoacetate of *cis*-cyclopent-2-ene-1,4-diol **49** has commonly been utilised for the enantioselective synthesis of carbocyclic nucleosides. Enzymatic hydrolysis of the *meso*-diacetate **48**, prepared in two steps from cyclopentadiene **36**, using *Candida antarctica* lipase B (Novo SP-435) gave the (1*S*,4*R*)-monoacetate (+)-**49** in high yield and optical purity (Scheme 1.23).[51,52]



Scheme 1.23 *Reagents and conditions:* a. CH_3CO_3H; b. $Pd(PPh_3)_4$; c. *Candida antarctica* lipase B, phosphate buffer (pH 8.0), 90%; d. *Candida antarctica* lipase B, isopropenyl acetate, 48%.

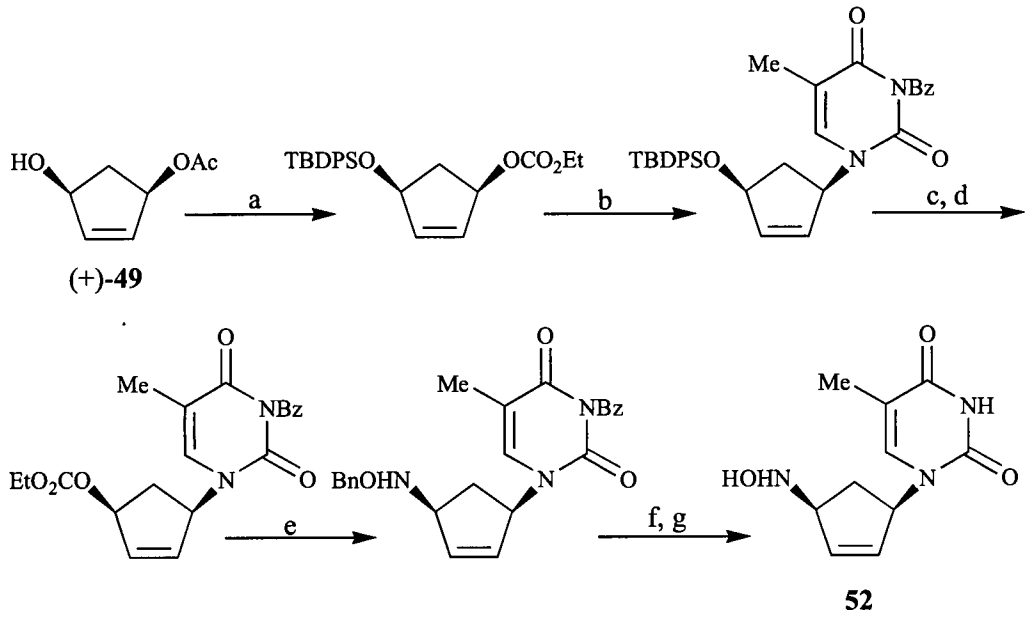
Enzyme catalysed transesterification of the *meso*-diol **50**, which was prepared by deacetylation of diacetate **48**, afforded the (1*R*,4*S*)-monoacetate (-)-**49** in poor yield but high optical purity (Scheme 1.23).[52]

Recently, Kobayashi *et al.*[53] have described a novel approach to (-)-aristeromycin **4**, which involves the nickel-catalysed coupling reaction of monoacetate (+)-**49** and lithium borates where the furyl group on the borate is transferred onto the monoacetate **49** to produce the *trans*-1,4-product **51** as illustrated in Scheme 1.24. The incorporation of the furyl group results in high stereoselectivity being obtained in the subsequent dihydroxylation step.



Scheme 1.24 *Reagents and conditions:* a. lithium 2-furylborate, $NiCl_2(PPh_3)_2$, 63%; b. $(PhO)_2P(O)N_3$, 75%; c. OsO_4, NMO, 72%; d. PPTS, $Me_2C(OMe)_2$, 92%; e. i. $RuCl_3$, $NaIO_4$; ii. CH_2N_2, 80%; f. $LiAlH_4$; g. standard adenine synthesis, 22% 2 steps; h. deprotection.

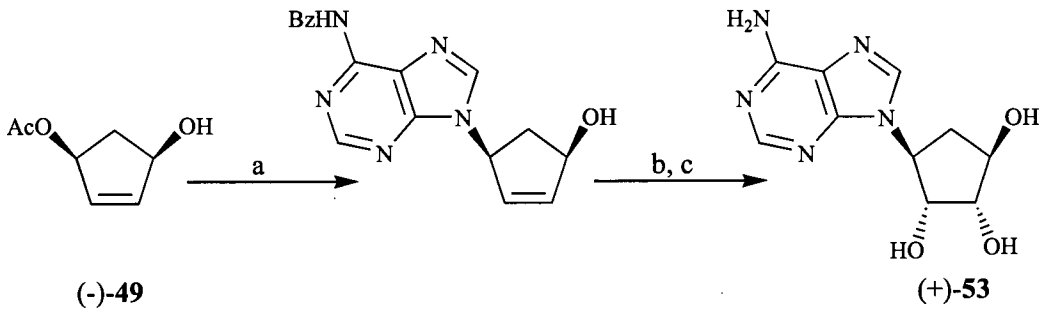
Matsuda and co-workers[54] have used the optically pure monoacetate (+)-**49** to synthesise carbocyclic nucleoside analogues **52** that have an hydroxyamino group at the carbocyclic moiety (Scheme 1.25).



Scheme 1.25 *Reagents and conditions:* a. i. TBDPSCl, imidazole, DMF, 0°C; ii. NaOMe, MeOH; iii. $ClCO_2Et$, pyridine, 97%; b. N3-benzoylthymine, $Pd_2(dba)_3.CHCl_3$, PPh_3, THF, 94%; c. TBAF, THF, quant.; d. $ClCO_2Et$, pyridine, quant.; e. NH_2OBn, HCl, $Pd_2(dba)_3.CHCl_3$, PPh_3, NaOH, THF, 94%; f. NaOMe, MeOH, 97%; g. BCl_3, CH_2Cl_2, 91%.

Compound **52** is a 4'-hydroxyaminated-carbocyclic equivalent of the anti-HIV agent 2',3'-didehydro-2',3'-dideoxythymidine (D4T). The synthesis of various uracil and cytidine derivatives of compound **52** was also achieved.[54]

The opposite enantiomer of the monoacetate (-)-**49** has been used to prepare (+)-5'-noraristeromycin **53**, which has been shown to possess anti-viral and anti-trypanosomal activity with no undesirable toxic side effects (Scheme 1.26).[55]

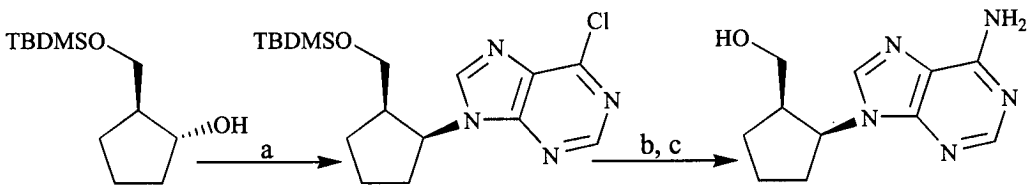


Scheme 1.26 *Reagents and conditions:* a. i. N6-benzoyladenine, NaH, DMSO; ii. $Pd(PPh_3)_4$, PPh_3, THF, 59%; b. NH_3, MeOH, 120°C, 80%; c. OsO_4, NMO, THF, H_2O, 55%.

This synthesis provides a more direct route to (+)-**53** than that previously described.[56]

(v) Synthesis from substituted cyclopentane systems

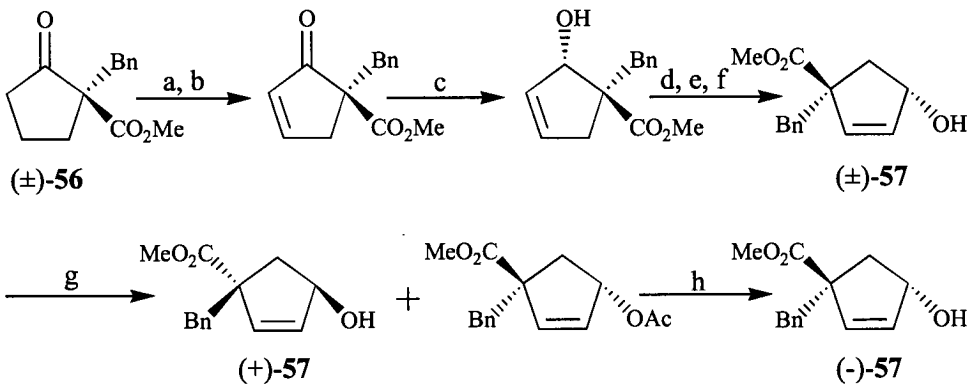
The silyloxy alcohol **54**, prepared from ethyl-2-oxocyclopentanecarboxylate by reduction followed by monosilylation, can be resolved by enzyme-catalysed transesterification with vinyl acetate in the presence of *Pseudomonas fluorescens* lipase to give both enantiomers in high optical purity.[57] These optically pure compounds have been used by Theil *et al.*[58] to prepare both enantiomers of the novel carbocyclic nucleoside **55** (Scheme 1.27).



(-)-**54** (-)-**55**

Scheme 1.27 *Reagents and conditions:* a. 6-chloropurine, DEAD, PPh_3, THF, 69%; b. AcOH, H_2O, THF, 95%; c. NH_3, MeOH, 86%.

Kato and co-workers[14] have recently reported the chemoenzymatic synthesis of optically active intermediates for the preparation of 4'-alkylcarbovir derivatives. The starting material for the synthesis was the cyclopentanone **56** which upon conversion to the racemic secondary alcohol (±)-**57** was subjected to enzymatic esterification to give both enantiomers of the key intermediate **57** as shown in Scheme 1.28.

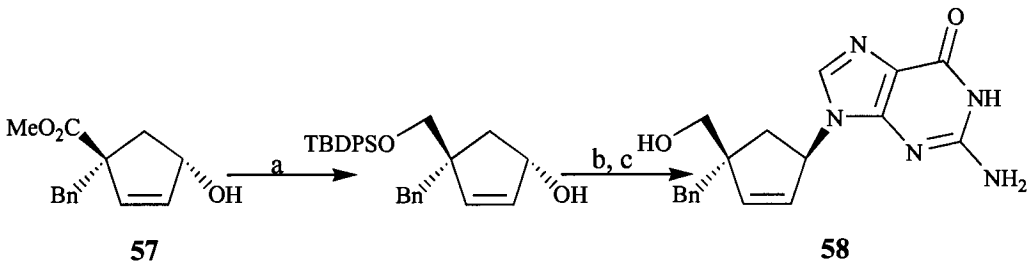


(±)-**56** (±)-**57**

(+)-**57** (-)-**57**

Scheme 1.28 *Reagents and conditions:* a. TMSOTf, Et_3N; b. $Pd(OAc)_2$, O_2, 91% 2 steps; c. $NaBH_4$, $CeCl_3$, 91%; d. Ac_2O, pyridine, 100%; e. $PdCl_2(MeCN)_2$, benzoquinone; f. K_2CO_3, MeOH, 100%; g. lipase "Amano" P, vinyl acetate; h. K_2CO_3, MeOH, 95%.

Either enantiomer of compound **57** can then be converted to the 4'-alkylcarbovir derivative **58** *via* a Mitsunobu reaction (Scheme 1.29).[14]



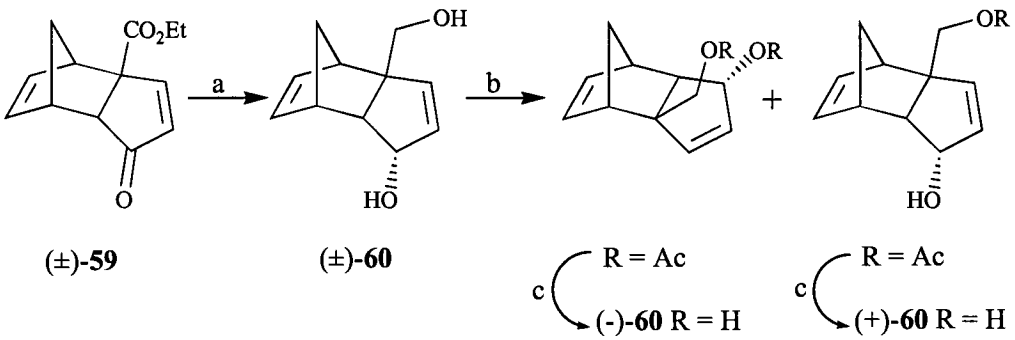
57 **58**

Scheme 1.29 *Reagents and conditions:* a. i. DHP; ii. $LiAlH_4$; iii. TBDPSCl; iv. PPTS, 72%; b. i. 2-amino-6-chloropurine, PPh_3, $EtO_2CN{=}NCO_2Et$; ii. TBAF, 36%; c. 1 M NaOH, 95%.

Intermediate (-)-**57** was also prepared by asymmetric synthesis, in which the key step was an asymmetric alkylation of a β-keto-ester.[59]

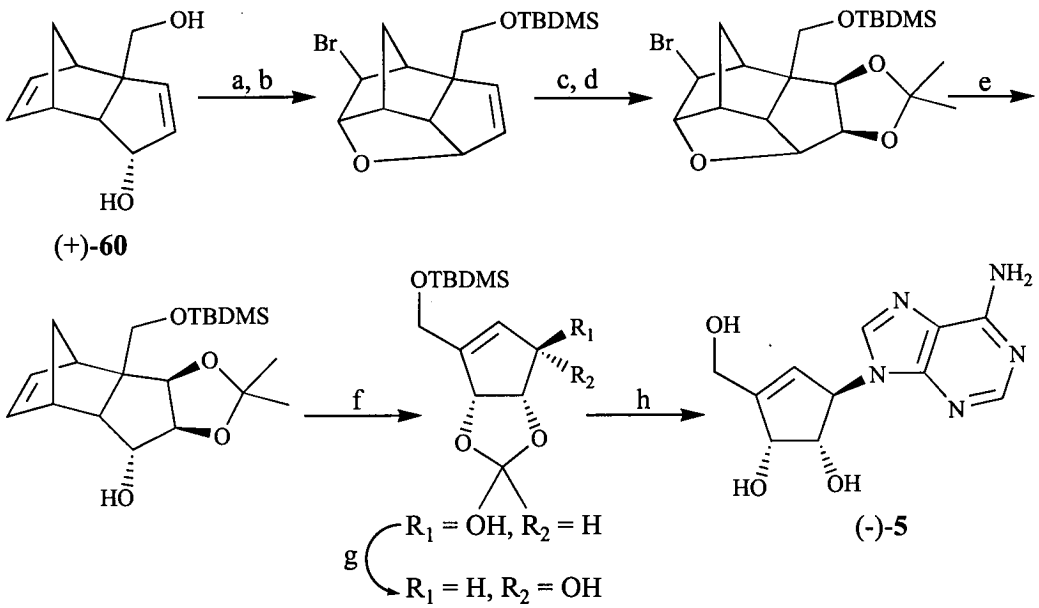
(vi) Synthesis from cyclic compounds derived from cyclopentadiene

Ogasawara and co-workers[60] have reported the concise synthesis of (-)-neplanocin A **5** from the tricyclic ester (±)-**59**, which is obtained from the Diels-Alder adduct of cyclopentadiene and benzoquinone.[61] The use of a lipase mediated kinetic resolution as shown in Scheme 1.30 gave both enantiomers of the diol (-)- and (+)-**60** in 41% yield, 92% e.e. and 46% yield, >99% e.e., respectively.[60]



Scheme 1.30 *Reagents and conditions:* a. DIBAL-H, toluene, -78°C, 65%; b. Lipase LIP, vinyl acetate, *t*-BuOMe; c. K_2CO_3, MeOH, 100%.

The optically pure diol (+)-**60** was then converted to (-)-neplanocin A **5** as described in Scheme 1.31.[60]



Scheme 1.31 *Reagents and conditions:* a. NBS, CH_2Cl_2, 100%; b. TBDMSCl, imidazole, DMF, 97%; c. OsO_4, NMO, aq. THF, 80%; d. DMP, PPTS, acetone, 100%; e. Zn, AcOH, MeOH, 95%; f. Ph_2O, reflux, 97%; g. i. PDC, CH_2Cl_2, 83%; ii. DIBAL-H, toluene, -78°C, 100%; h. i. adenine, PPh_3, THF, diisopropyl azodicarboxylate, 84%; ii. HCl, MeOH, 90%.

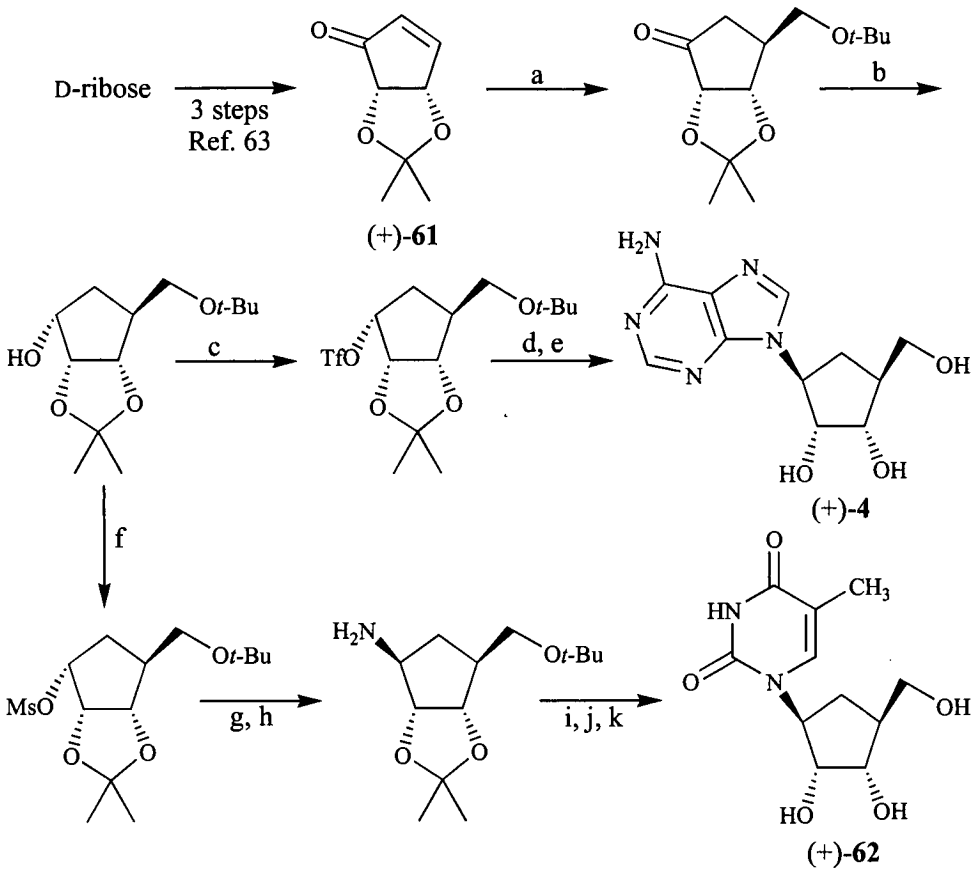
(b) Synthesis from the chiral pool

Many recent syntheses of carbocyclic nucleosides have been accomplished by using starting materials from the chiral pool.

(i) Synthesis from carbohydrates

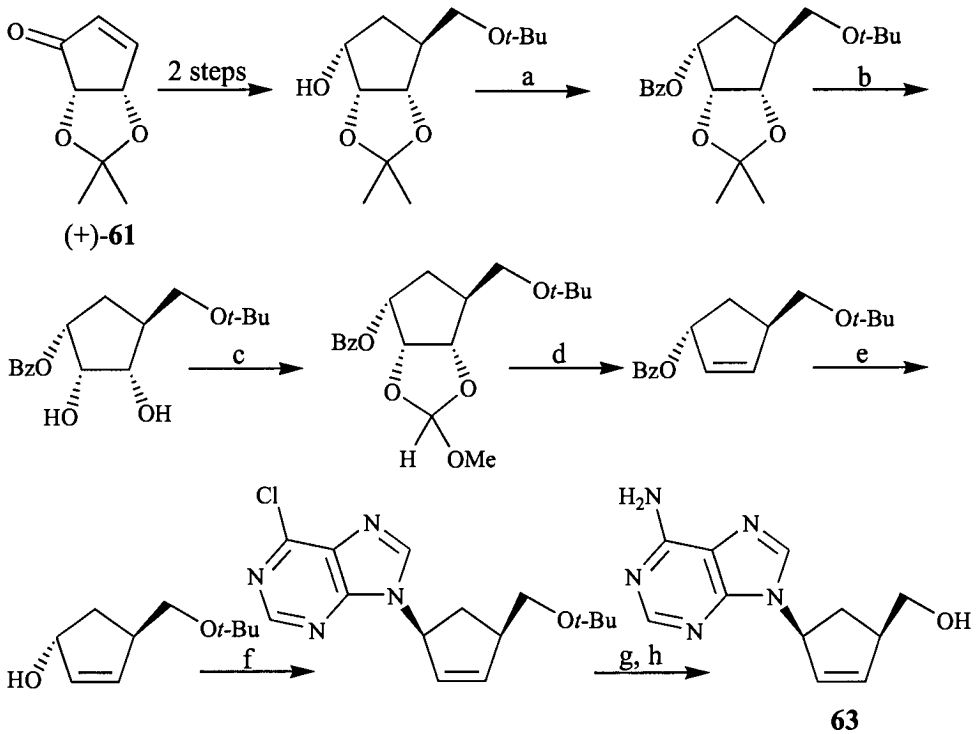
The use of carbohydrates and their derivatives as chiral building blocks for the synthesis of enantiomerically pure non-carbohydrate compounds continues to be an area of active investigation, particularly for preparing carbocyclic nucleosides.

The first asymmetric synthesis of L-cyclopentyl carbocyclic nucleosides was reported by Chu *et al.*,[62] who reported the synthesis of (+)-β-L-aristeromycin **4** and its thymine analogue **62** from the (+)-cyclopentenone **61** which can be prepared from D-ribose (Scheme 1.32).[63]



Scheme 1.32 *Reagents and conditions:* a. $(t\text{-BuOCH}_2)_2\text{CuLi}$, *t*-BuOMe, THF, -30°C, 87%; b. DIBAL-H, CH_2Cl_2, -78°C, 82%; c. $(CF_3SO_2)_2O$, pyridine, 0°C, 97%; d. adenine, NaH, 18-crown-6, DMF, 0-20°C, 32%; e. CF_3CO_2H:H_2O (2:1), 50°C, 80%; f. CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0°C, quant.; g. LiN_3, DMF, 140°C, 89%; h. 5% Pd/C, EtOH; i. β-methoxy-α-methacryloyl isocyanate, DMF, -20°C to 20°C, 88% 2 steps; j. 30% NH_4OH, EtOH, 80-100°C, 85%; k. CF_3CO_2H:H_2O (2:1), 50°C, 79%.

Chu and co-workers[64] have also described the synthesis of carbocyclic β-L-2',3'-didehydro-2',3'-dideoxyadenosine (D4A) **63** and its analogues, again starting from the known (+)-cyclopentenone **61**, prepared as previously from D-ribose (Scheme 1.33).[63]

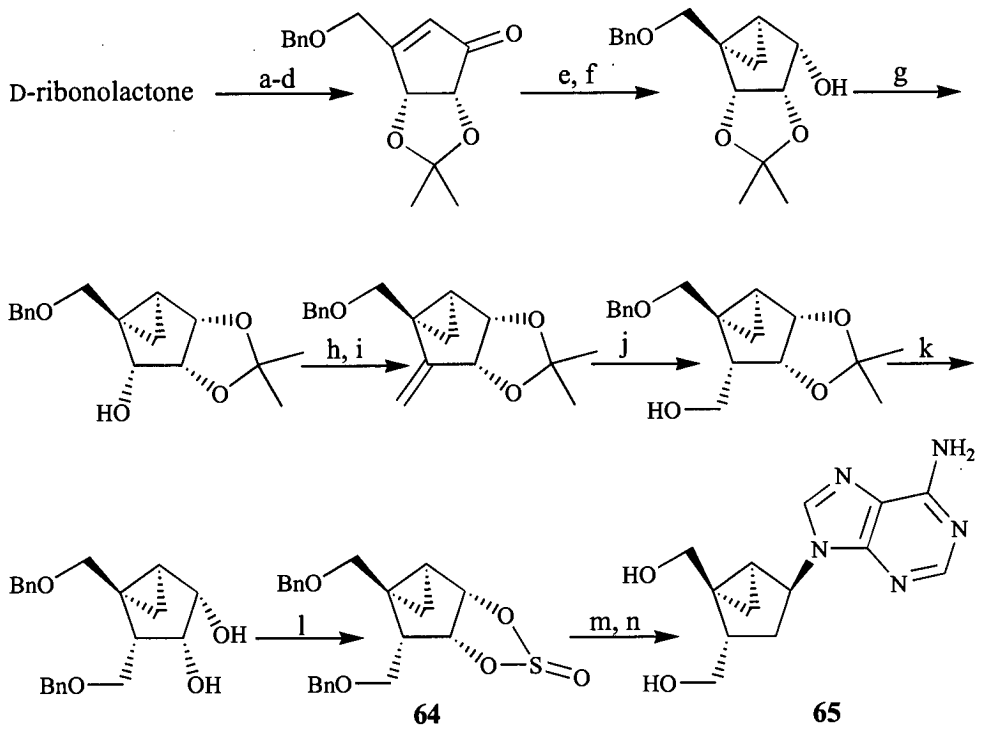


Scheme 1.33 *Reagents and conditions:* a. BzCl, pyridine, 93%; b. conc. HCl, MeOH, 93%; c. $CH(OMe)_3$, PPTS; d. Ac_2O, 120-130°C, 68% 2 steps; e. 2 M NaOH, MeOH, 93%; f. 6-chloropurine, PPh_3, DEAD, dioxane, 35%; g. NH_3, MeOH, 80-90°C, 83%; h. CF_3CO_2H:H_2O (2:1), 50°C, 93%.

L-Carbocyclic nucleosides are of particular interest as a number of L-nucleosides have proven to be of great importance as anti-viral and anti-tumour agents. Some of these L-nucleosides are more potent and less toxic than their D-counterparts.[62,64]

The commercially available D-ribonolactone is also a useful starting material for the synthesis of optically pure carbocyclic nucleosides. Jeong *et al.*[18] have used this compound for the preparation of the novel cyclopropyl-fused carbocyclic nucleoside **65**, in which the bicyclic system was introduced by Simmons-Smith cyclopropanation as shown in Scheme 1.34.

Bicyclic carbocyclic nucleosides are of interest because if the orientation of the fused ring is correct, its effect is to freeze the conformation of the 5-membered ring into that required for optimum binding to enzyme substrates and therefore improve the biological activity.[65]

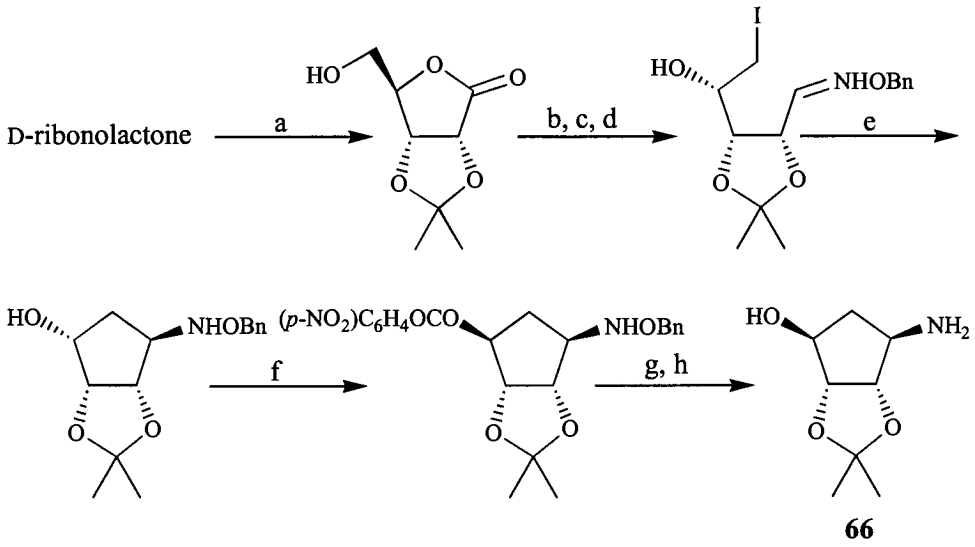


Scheme 1.34 *Reagents and conditions:* a. $LiCH_2P(O)(OCH_3)_2$; b. $NaOCH_3$; c. CrO_3-pyridine; d. K_2CO_3, 18-crown-6; e. $NaBH_4$, $CeCl_3$; f. Zn/Cu, CH_2I_2, 73%; g. PTSA, acetone, 56°C, 57%; h. TPAP, NMO, CH_2Cl_2, 100%; i. $CH_3P(C_6H_5)_3Br$, *n*-BuLi, THF, 0°C, 90%; j. BH_3-THF, $NaBO_3$-H_2O, 99%; k. i. BnBr, NaH; ii. 1 M HCl, MeOH-THF, 88%; l. Et_3N, $SOCl_2$, 0°C, 80%; m. adenine, NaH, DMF, 18-crown-6, 50%; n. i. CS_2, CH_3I, NaH; ii. Et_3B, *n*-Bu_3SnH; iii. Pd black, HCO_2H, 61%.

The syntheses of the uracil, cytosine and guanosine derivatives of compound **65** *via* the cyclic sulfite **64** are also described.[18]

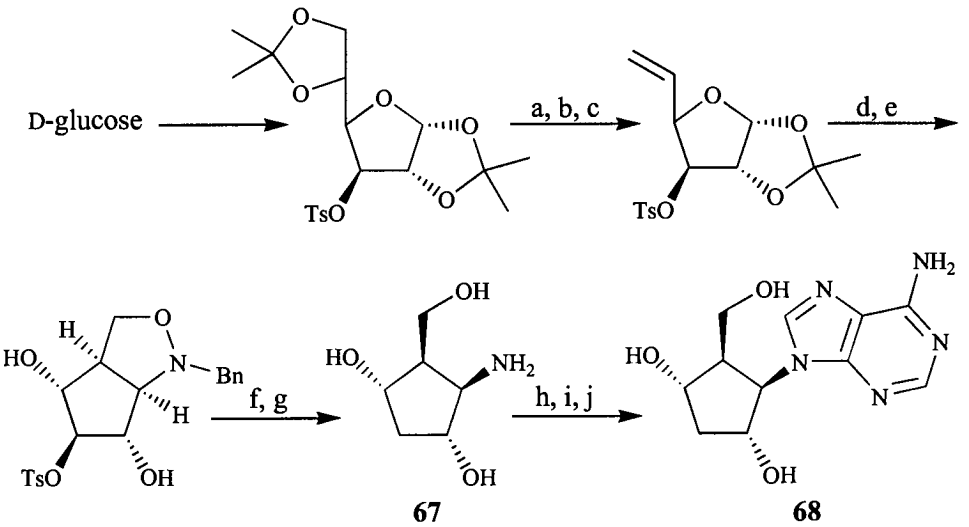
A novel method for the preparation of aminocyclopentanetriol **66** from D-ribonolactone, in which the key step is a tributyltin hydride mediated free radical cyclisation, has been reported by Marco-Contelles *et al.* (Scheme 1.35).[66]

The purine or pyrimidine base can then be constructed onto intermediate **66** by standard procedures to give the corresponding 5'-norcarbocyclic nucleoside analogues.



Scheme 1.35 *Reagents and conditions:* a. acetone, PTSA, quant.; b. I_2, PPh_3, imidazole, 50%; c. DIBAL-H, toluene, -78°C, 77%; d. $BnONH_3Cl$, pyridine, 76%; e. *n*-Bu_3SnH, AIBN, toluene, 75%; f. PPh_3, DEAD, *p*-nitrobenzoic acid, 78%; g. $NaOCH_3$, MeOH, 90%; h. LiAlH, THF, 58%.

As a carbohydrate precursor D-glucose is very useful due to its ready availability.

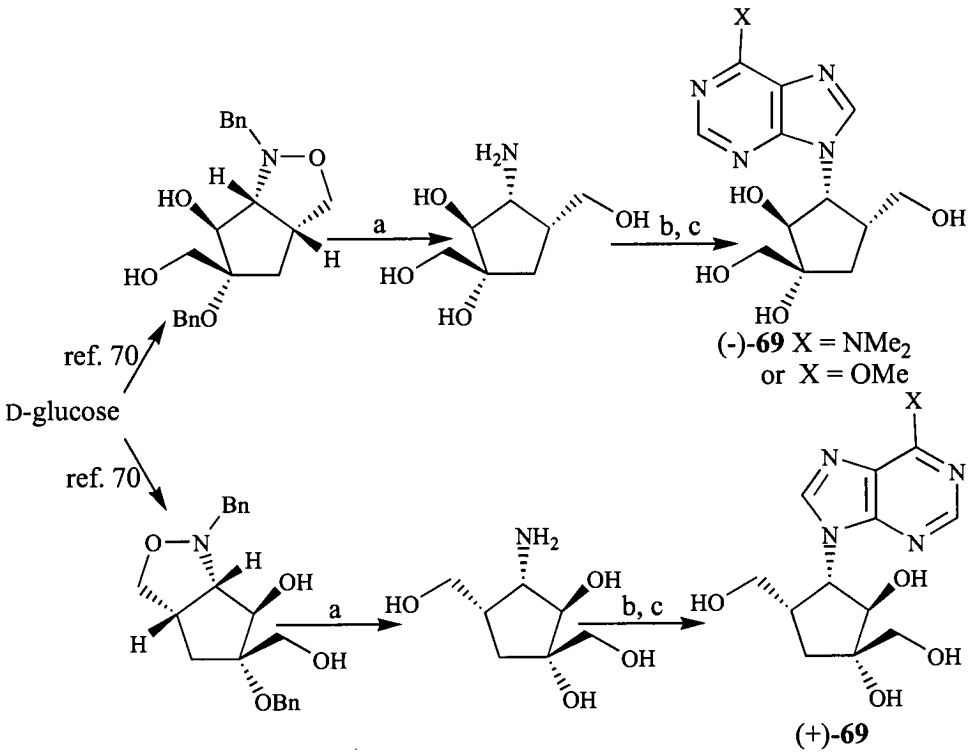


Scheme 1.36 *Reagents and conditions:* a. AcOH, 55°C; b. $NaIO_4$, EtOH; c. $Ph_3P^+CH_3I^-$, *n*-BuLi, -60°C, 66%; d. H_2SO_4 in CH_3CN-H_2O, 60-65°C; e. $PhCH_2NHOH$, EtOH, 74%; f. $LiAlH_4$, THF, reflux; g. Pd/C, cyclohexene, EtOH, 91%; h. 5-amino-4,6-dichloropyrimidine, Et_3N, *n*-BuOH, reflux, 43%; i. $HC(OEt)_3$, PTSA, DMF, 44%; j. NH_3, MeOH, 100°C, 80%.

Mandal and co-workers[67] have recently reported the use of an intramolecular nitrone cycloaddition (INC) for converting D-glucose into enantiomerically pure functionalised five-, six-, and seven- membered carbocycles. Using this

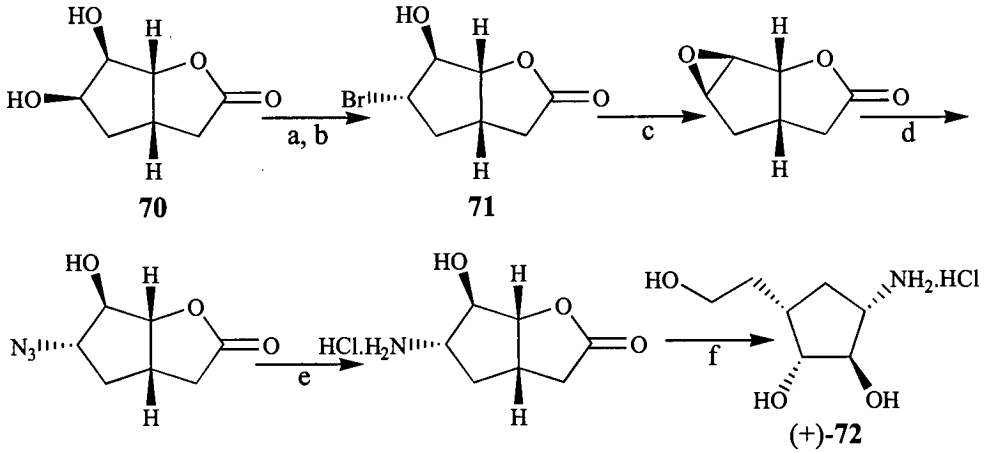
methodology the five-membered chiral trihydroxy-aminocyclopentane **67** has been efficiently synthesised and converted to the aristeromycin analogue **68** as described in Scheme 1.36.[68]

Mandal[69] has also described a simple method for the preparation of the pair of enantiomers of the carbocylic nucleoside **69** starting with enose-nitrones derived from D-glucose (Scheme 1.37).



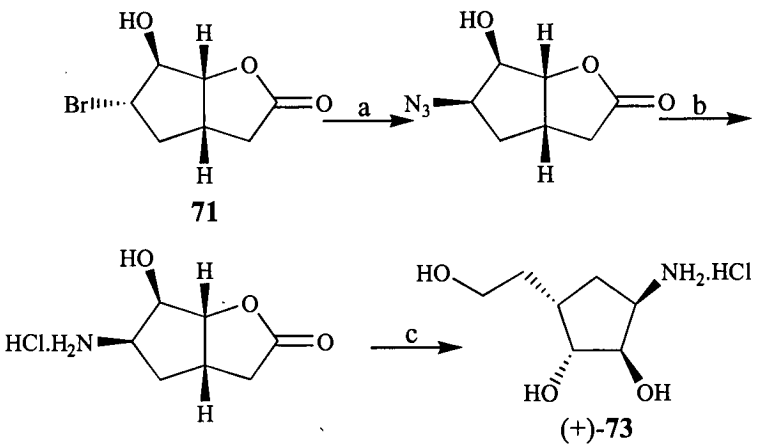
Scheme 1.37 *Reagents and conditions:* a. 10% Pd/C, EtOH, reflux, 88%; b. 5-amino-4,6-dichloropyrimidine, Et_3N, *n*-BuOH, reflux, 80%; c. $HC(OEt)_3$, PTSA, DMF, 20°C, 53%.

Lundt *et al.*[71] have reported the synthesis of the polyhydroxylated aminocyclopentane **72** from the bicyclic lactone **70** as shown in Scheme 1.38. The bicyclic lactone **70** was prepared by stereoselective radical induced carbocyclisation of ω-bromo-α-,β-unsaturated heptonolactones.[72]



Scheme 1.38 *Reagents and conditions:* a. i. HBr, AcOH; ii. Ac_2O, 85%; b. HCl, MeOH, quant.; c. K_2CO_3, acetone, 97%; d. NaN_3, NH_4Cl, DMF, 90°C, 73%; e. H_2, Pd/C, HCl, EtOH, 67%; f. $Ca(BH_4)_2$, EtOH, 57%.

Lundt[71] also described the synthesis of a stereoisomer **73** of aminocyclopentane **72** from bromohydrin **71** as shown in Scheme 1.39.



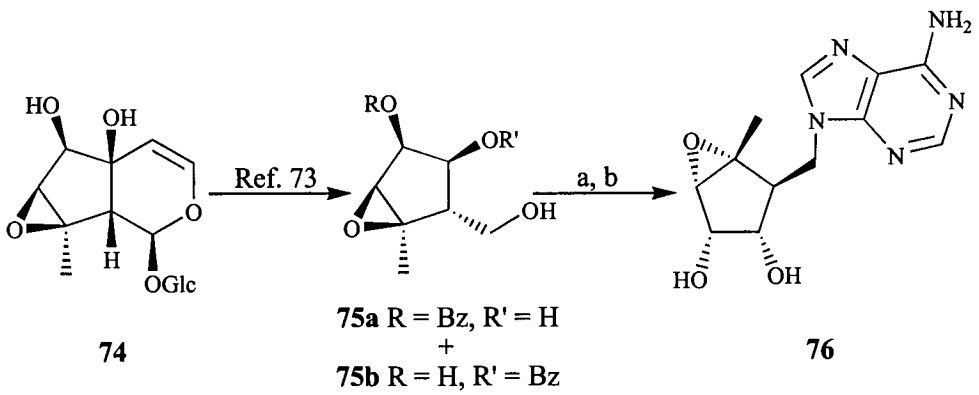
Scheme 1.39 *Reagents and conditions:* a. NaN_3, DMF, 80°C, 35%; b. H_2, Pd/C, HCl, EtOH, quant.; c. i. HMDS, TMSCl, CH_3CN, reflux; ii. $BH_3.SMe_2$, dioxane, 76%.

The aminocyclopentanes **72** and **73** can be converted into carbocyclic nucleosides by standard procedures.

(ii) Synthesis from iridoids

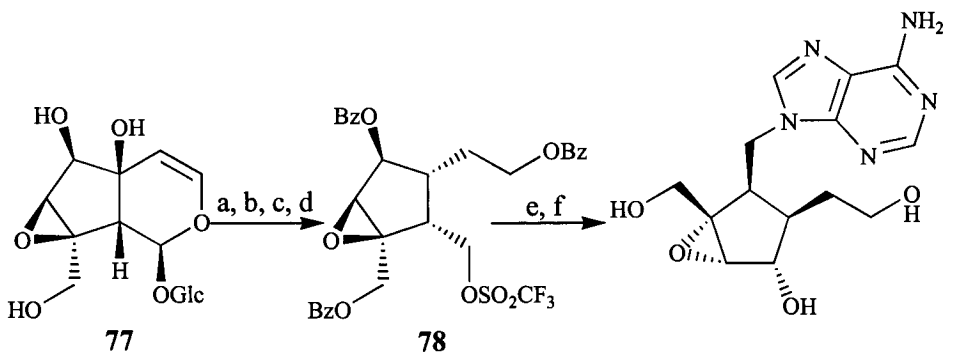
Franzyk *et al.*[15] have used chiral building blocks derived from the iridoid glucosides, antirrhinoside **74** and catalpol **77** to prepare optically pure carbocyclic nucleosides *via* polysubstituted cyclopentylmethanols. The interconvertible cyclo-

pentylmethanols **75a** and **b** were synthesised in 5 steps from antirrhinoside **74** before being converted *via* a Mitsunobu reaction to the carbocyclic nucleoside **76** as outlined in Scheme 1.40.[15]



Scheme 1.40 *Reagents and conditions:* a. 6-chloropurine, PPh_3, DEAD, THF; b. NH_3, 45°C, 41% 2 steps.

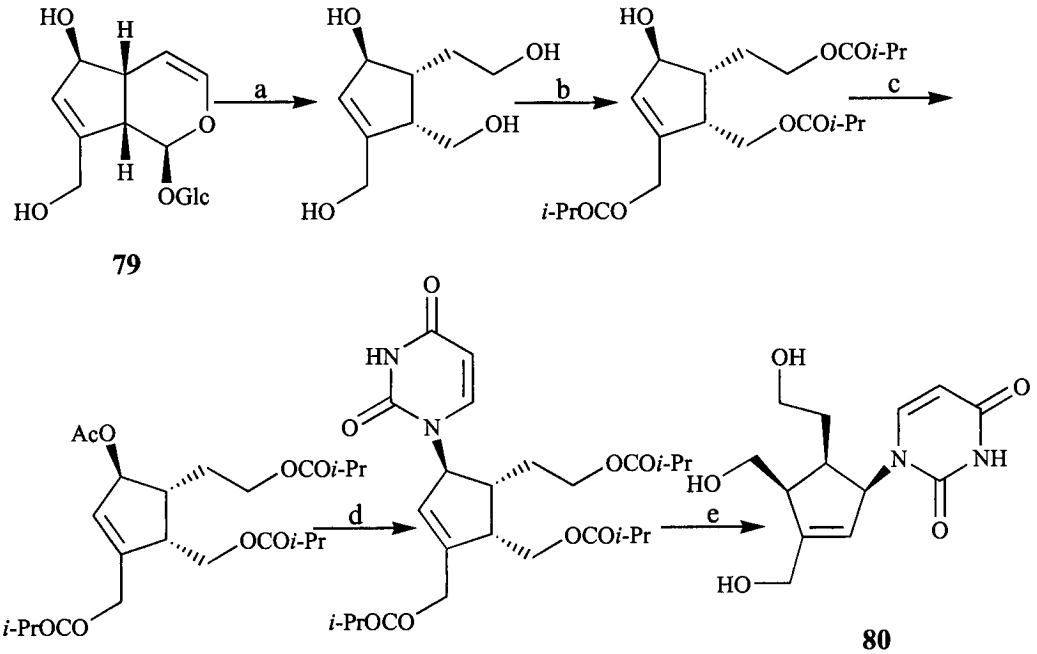
Franzyk[15] also reported the synthesis of the functionalised cyclopentane **78** from the iridoid glucoside, catalpol **77**. In this case the heterocyclic base was incorporated directly *via* displacement of a triflate as shown in Scheme 1.41.



Scheme 1.41 *Reagents and conditions:* a. β-glucosidase, H_2O; b. $NaBH_4$, 82% 2 steps; c. BzCl, pyridine, CH_2Cl_2, -78°C, 60%; d. $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2; e. 6-iodopurine (Bu_4N^+ salt), CH_2Cl_2, -10°C, 73%; f. NH_3, 45°C, 86%.

(iii) Synthesis from monoterpenoids

Bianco and co-workers[74] have reported the synthesis of a novel carbovir analogue **80** from the monoterpenoid aucubin **79**, a methyl-cyclopentanoid glucoside present in large quantities in plants of *Aucuba* genus (Scheme 1.42).



Scheme 1.42 *Reagents and conditions:* a. i. $Hg(OAc)_2$, $NaBH_4$ or ii. β-glucosidase, $NaBH_4$, 90%; b. Isobutyric anhydride, pyridine, 90%; c. Ac_2O, pyridine, 99%; d. i. uracil, CH_3CN, TMSCl, HMDS; ii. $SnCl_4$, 56%; e. DIBAL-H, CH_2Cl_2, -78°C, 95%.

1.2.3 Summary

The synthesis of enantiomerically pure carbocyclic nucleosides has advanced in recent years with a variety of new, efficient approaches being developed. The use of syntheses which rely on enzymatic resolution have been particularly useful especially with the recent low cost, commercial availability of the key chiral intermediates. However, new, more efficient syntheses are still required because of the great demand for anti-viral and anti-tumour agents.

A further area of interest is the biosynthesis of the naturally occurring carbocyclic nucleosides aristeromycin **4** and neplanocin A **5** by the organism *Streptomyces citricolor*. Elucidation of the biosynthetic pathway would allow for the preparation of novel analogues of carbocyclic nucleosides, whilst selective blocking of the pathway could provide useful chiral intermediates that would be difficult to prepare by alternative chemical procedures. The studies which have been carried out towards the elucidation of this pathway will be discussed in Section 1.3.

1.3 The Naturally Occurring Carbocyclic Nucleosides Aristeromycin and Neplanocin A

Aristeromycin **4**, which is the carbocyclic analogue of adenosine, was first isolated in 1967 from the fermentation broth of *Streptomyces citricolor* having been previously synthesised in racemic form.[2,75] Subsequently in 1981 neplanocin A **5** and some closely related compounds were isolated from cultures of *Ampullariella regularis* and more recently neplanocin A has been shown to be co-produced alongside aristeromycin by *Streptomyces citricolor*.[3,76]

As a result of their close structural relationship to natural nucleosides, aristeromycin and neplanocin A have been shown to possess potent biological activity.[2,3] For example, neplanocin A displays anti-viral and anti-tumour activity and both aristeromycin and neplanocin A have been shown to inhibit the enzyme S-adenosyl-L-homocysteine hydrolase, which plays an important role in regulating biological methylation reactions.[77]

Due to their potential as therapeutic agents, aristeromycin and neplanocin A have attracted considerable synthetic interest which includes several reports of their total syntheses, the most recent of which have been described in Section 1.2.

1.3.1 The biosynthesis of aristeromycin and neplanocin A by *Streptomyces citricolor*

The biosynthesis of aristeromycin and neplanocin A first received attention in the mid 1980's by R. J. Parry *et al.* and through a combination of their work and from more recent studies carried out by N. J. Turner *et al.* the pathway shown in Figure 1.2, for the biosynthesis of aristeromycin **4** and neplanocin A **5** from D-glucose **81** by *Streptomyces citricolor*, has been established.[78]

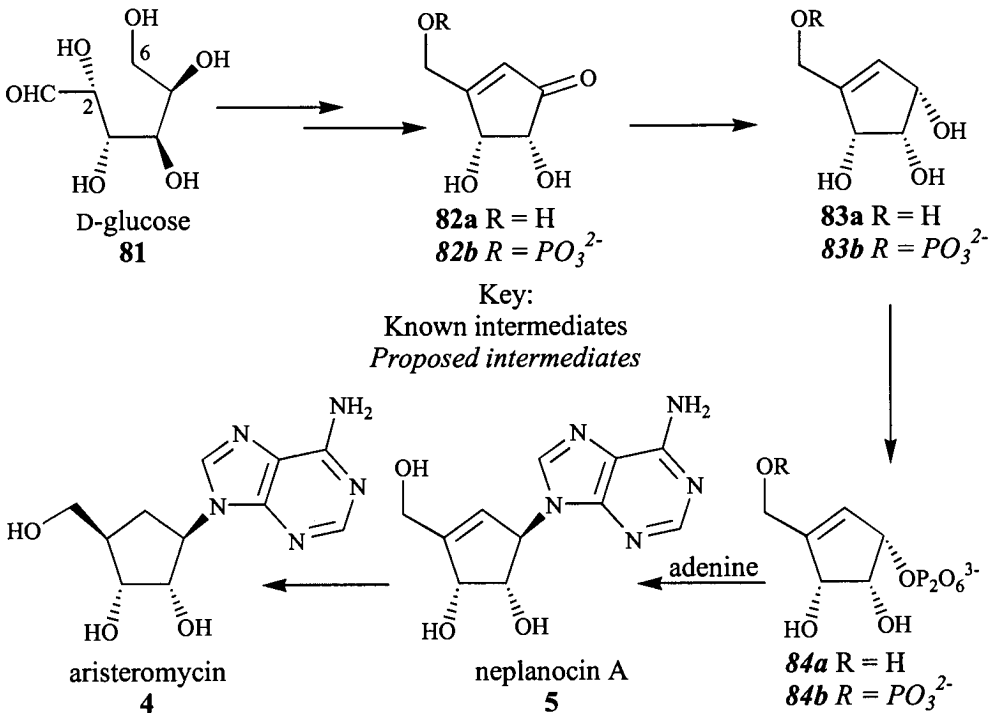


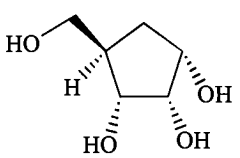
Figure 1.2 Proposed pathway for the biosynthesis of aristeromycin and neplanocin A by *Streptomyces citricolor*

The studies that have led to this proposed biosynthetic pathway are outlined below.

(a) Origin of the cyclopentane ring

Early biosynthetic studies carried out by Parry *et al.* established that the carbocyclic skeleton of **4** and **5** was derived from D-glucose **81**.[76,79] Through a series of feeding experiments using isotopically labelled precursors, it was established that formation of the carbocyclic ring in aristeromycin results from C-C bond formation between the C-2 and C-6 carbon atoms of D-glucose **81**. Additionally, Parry deduced that the cyclisation proceeded with loss of the 6-*pro*-S hydrogen atom.[76]

In addition to these initial feeding experiments, Parry and Johnson reported the preparation of the putative intermediates **85** and **86**, and by isotope dilution studies, concluded that the saturated tetrol **85** and the aminotriol **86** might be intermediates on the biosynthetic pathway.[80,81]



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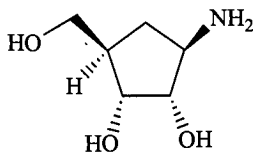


86

Work within the Turner group involved the use of a series of mutants of *Streptomyces citricolor* developed by GlaxoWellcome, that were blocked in their ability to synthesise either neplanocin A or aristeromycin. However, the production of aristeromycin and neplanocin A could be rescued by combinations of certain mutants in which the supernatant from cultures of one mutant (secretor) were added to a culture of a second mutant (converter). Such cosynthesis experiments identified a secretor/converter pairing in which mutant CC914 secreted a compound that supported the production of neplanocin A and aristeromycin in a second mutant CC940. The structure of this compound was determined to be the unsaturated tetrol **83a** as shown in Figure 1.3.[82]

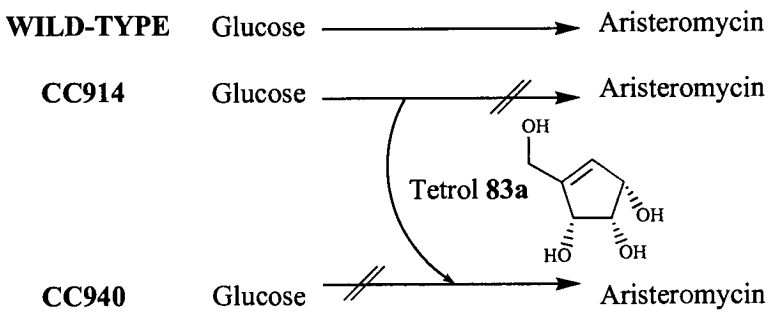
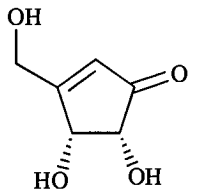


Figure 1.3 Cosynthesis experiments using mutants of *Streptomyces citricolor*

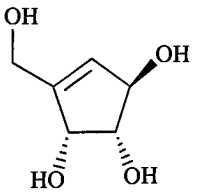
Further evidence for the participation of tetrol **83a** in the biosynthesis was obtained by ^{13}C-isotopic labelling experiments.[83]

Further putative intermediates were synthesised and fed to the converter mutant CC940. Those compounds that supported aristeromycin production were deemed to lie on the biosynthetic pathway. Through these studies it was established that the cyclopentenone **82a** was an intermediate on the biosynthetic pathway. However, when the C-1-*epi*-tetrol **87**, aminotriol **88**, saturated tetrol **85** and aminotriol **86** were prepared and fed to converter mutant CC940, there was no production of aristeromycin or neplanocin A.[83] These observations suggest that contrary to the

previous proposal by Parry and Johnson, the saturated tetrol **85** and aminotriol **86** do not lie on the central biosynthetic pathway.



82a



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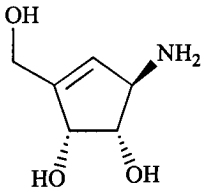
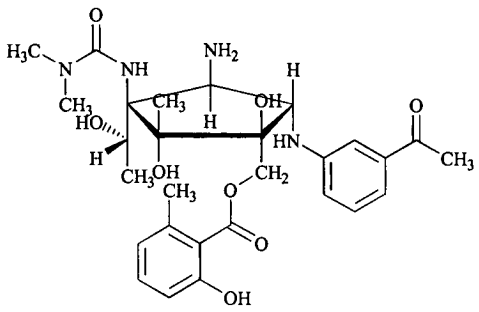


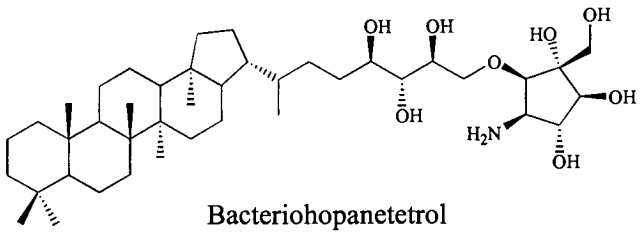
88

(b) Mechanism of conversion of D-glucose to the cyclopentane ring

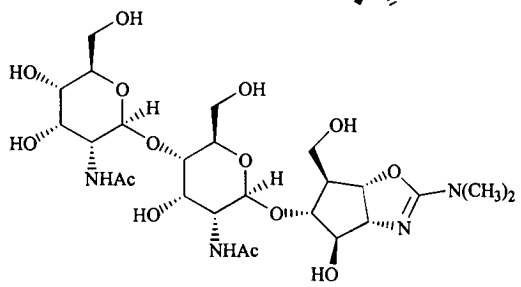
The processes by which nature is able to convert carbohydrates to 6-membered carbocyclic rings is now well understood owing to the detailed studies that have been carried out on the biosynthesis of inositols and shikimic acid.[78] By comparison, very little is known about the way in which carbohydrates are enzymatically converted to 5-membered carbocyclic rings. There are only four examples in the literature, including aristeromycin and neplanocin A, for which any biosynthetic studies have been reported. Pactamycin **89** which has been isolated from *Streptomyces pactam* var. *pactam*; bacteriohopanetetrol **90** which was isolated from *Methylobacterium organophilum*, *Rhodopseudomonas acidophilia*, and *Zymomonas mobilis*; and allosamidin **91** isolated from a *Streptomyces* sp. However, in contrast to the biosynthesis of neplanocin A and aristeromycin where the carbocyclic ring is formed between C-2 and C-6 of D-glucose; in the case of pactamycin **89**, bacteriohopanetetrol **90** and allosamidin **91**, the carbocyclic ring is formed between C-1 and C-5 of D-glucose/glucosamine.[78]



Pactamycin
89



Bacteriohopanetetrol
90



Allosamidin
91

By analogy with the shikimate biosynthetic pathway,[78] it is possible to suggest the route from D-glucose **81** to the cyclopentenone **82a** shown in Figure 1.4.

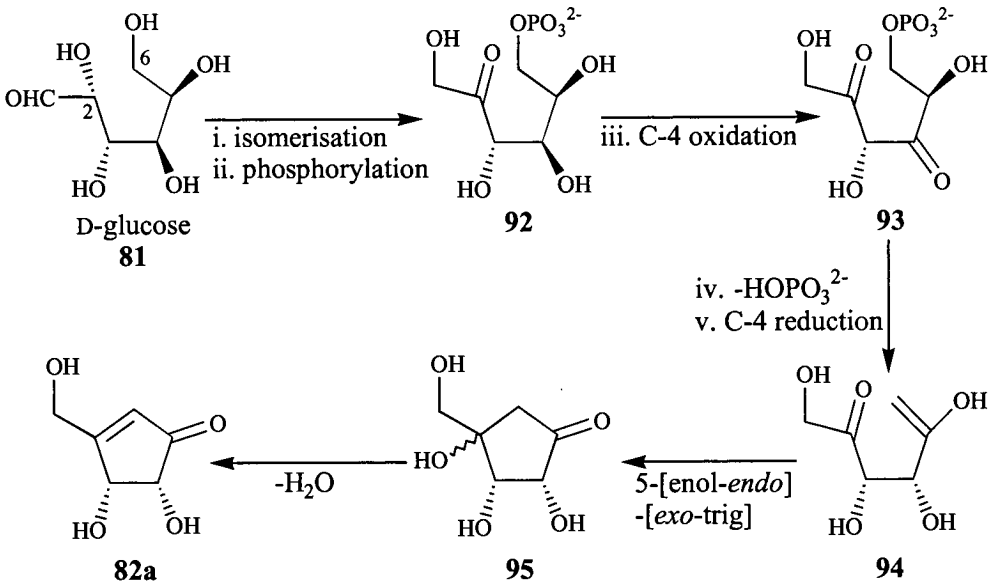


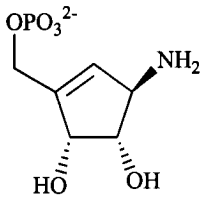
Figure 1.4 Proposed route for the conversion of D-glucose to the carbocyclic ring

In this route D-glucose **81** is converted to fructose-6-phosphate **92** which undergoes oxidation at C-4 to give **93**. Elimination of phosphate from **93** followed by reduction at C-4 yields the enol **94** which would undergo an intramolecular aldol cyclisation to give the keto-tetrol **95** which would then undergo elimination to cyclopentenone **82a**, which is a known intermediate on the biosynthetic pathway.

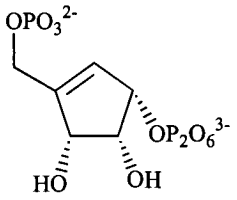
Indirect evidence has been obtained for the possible participation of some of the intermediates between **81** and **82a**.[84] Direct evidence could be obtained by the synthesis of keto-tetrol **95**, phosphates **92** and **93**, and diketone **94** and feeding to converter mutants of *Streptomyces citricolor*.

(c) Origin of the adenine base

By comparison to previously published work on purine biosynthesis,[85] isotopic labelling studies carried out by Parry's group[76] suggested that the adenine ring is derived from *de novo* purine biosynthesis *i.e.* the adenine ring is biosynthesised by stepwise construction of the adenine ring on a carbocyclic analogue of 5-phosphoribosyl pyrophosphate containing a pre-existing amino group at C-1 (**96**). However, some evidence was obtained for the operation of a salvage pathway in which the adenine ring is incorporated intact *i.e.* the adenine ring is incorporated intact to a carbocyclic intermediate activated at C-1 by a pyrophosphate group (**84b**).



96



84b

In order to probe further the origin of the adenine ring, studies carried out by Turner *et al.*[83] centred on the isolation of mutants of *Streptomyces citricolor* that were defective in purine biosynthesis and required exogenous addition of adenine in order for normal growth to occur. One such mutant, CC268, was grown on a defined medium containing 8-^{13}C-adenine as the only source of purines. The

labelled aristeromycin that was produced contained the ^{13}C label only at C-8 in the adenine ring (approx. 75% incorporation) as shown in Figure 1.5, therefore establishing the existence of a pathway in which adenine can be incorporated intact into aristeromycin. However, it was also shown that 1-^{13}C glycine could function as a precursor leading to the production of 4-^{13}C aristeromycin (approx. 20% incorporation) as shown in Figure 1.5. This observation may be due to some "leakiness" of the adenine auxotroph and is consistent with either a *de novo* or salvage-type pathway.

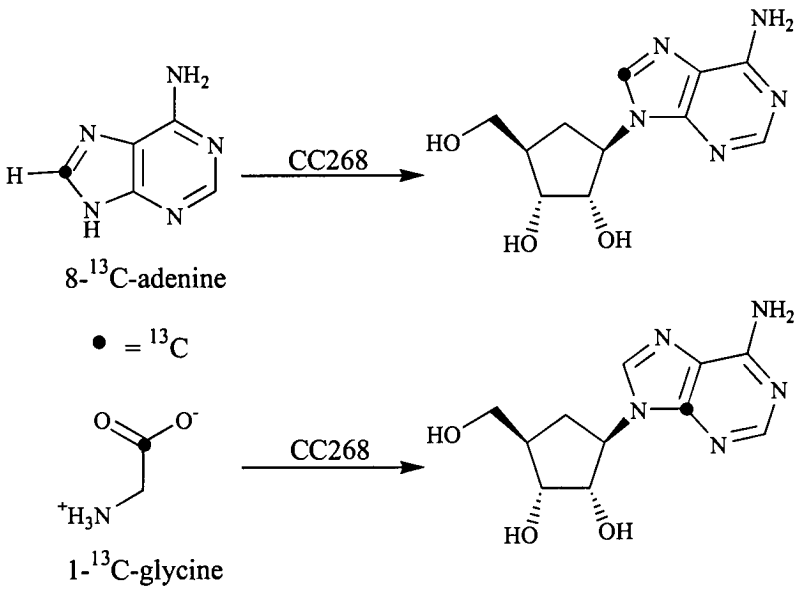


Figure 1.5 Origin of the adenine base in aristeromycin

Further evidence for the operation of a salvage-type pathway has been obtained by recent studies carried out within the Turner group.[86] ^{14}C-labelled adenine together with tetrol **83a** were fed to the converter mutant CC940. After incubation at 30°C for 24 hours, the incorporation of labelled adenine into neplanocin A (3.6% based on radioactive yield) was suprisingly high given the level of incorporation previously reported by Parry.[76]

These results strongly support the direct incorporation of adenine as the major route to aristeromycin, whilst the involvement of a *de novo* pathway cannot be completely eliminated. In addition, direct incorporation of the adenine base *via* the pyrophosphate **84b** would be favoured mechanistically due to displacement at an allylic centre.

(d) Conversion of neplanocin A to aristeromycin

The co-production of neplanocin A and aristeromycin in cultures of the wild-type *Streptomyces citricolor* suggested that these two metabolites, differing only in the presence of a double bond between C-4' and C-6', might be closely related on the biosynthetic pathway. Furthermore, mutant strains were isolated which produce increased levels of neplanocin A relative to aristeromycin. Preliminary experiments had also shown that a number of mutants which were unable to produce either aristeromycin or neplanocin A, were able to convert added neplanocin A into aristeromycin.[82] However, the observations of Parry, in which evidence for the participation of the saturated intermediates **85** and **86** was obtained, suggested that neplanocin and aristeromycin might be biosynthesised along independent parallel biosynthetic routes.[80,81]

In order to establish whether or not the normal biosynthetic route to aristeromycin by *Streptomyces citricolor* proceeded *via* neplanocin A by saturation of the double bond, studies within the Turner group were stimulated by an earlier experiment reported by Parry.[79] Using wild-type *Streptomyces citricolor*, Parry showed that 6-$^{2}H_{2}$-D-glucose was converted to 6'-^{2}H-aristeromycin in which the deuterium atom was located only in the 6'-*pro-S* site as shown in Figure 1.6.

Furthermore, by feeding tritium labelled forms of D-glucose Parry concluded that the cyclisation reaction proceeded with loss of the 6-*pro-S* hydrogen atom of D-glucose and that the 6-*pro-R* atom of D-glucose became the 6'-*pro-S* hydrogen atom in aristeromycin.

An analogous experiment was carried out by Turner *et al.*[83] using a mutant of *Streptomyces citricolor* (CC1026) which produced neplanocin A but no aristeromycin. Administration of 6-$^{2}H_{2}$-D-glucose to the CC1026 mutant gave 6'-^{2}H-neplanocin A. This material was then fed to a second mutant (CC826) which was blocked in the production of both aristeromycin and neplanocin A and was also able to act as an efficient converter of neplanocin A to aristeromycin. The 6'-^{2}H-aristeromycin isolated from this experiment was stereochemically identical to that derived from the 6-$^{2}H_{2}$-D-glucose experiment as shown in Figure 1.6, which suggested that neplanocin A is the direct precursor of aristeromycin.

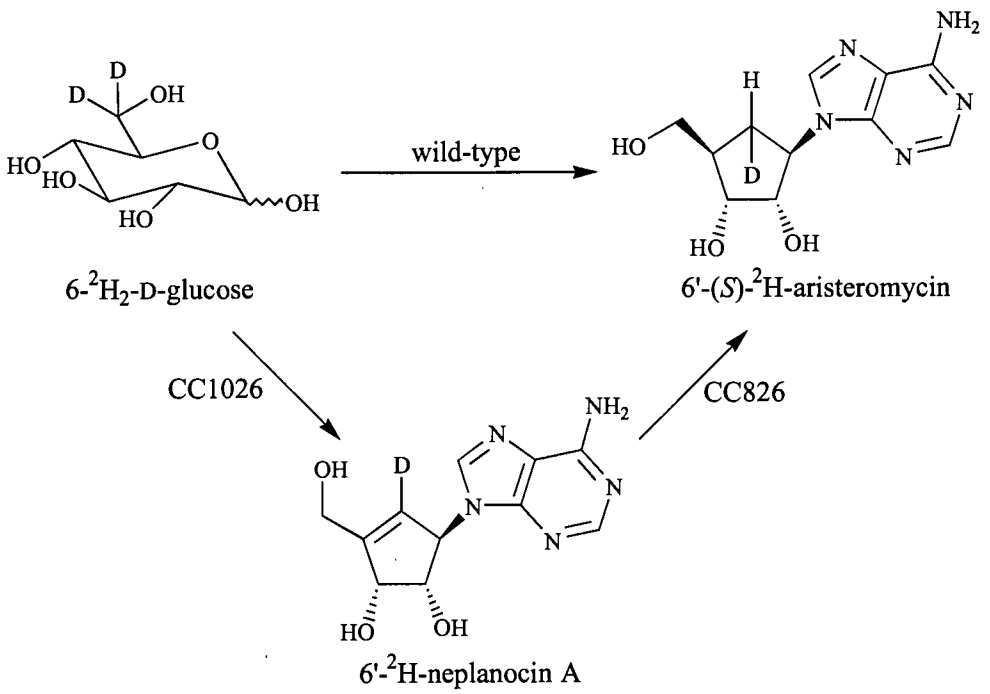


Figure 1.6 Stereochemistry of deuterium incorporation into aristeromycin

Further evidence for the direct conversion of neplanocin A to aristeromycin was obtained by Parry using cell-free extracts of wild-type *Streptomyces citricolor*.[87] Parry demonstrated that a partially purified cell-free extract was able to catalyse the NADPH-dependent reduction of neplanocin A to aristeromycin. Furthermore, it was shown that the reaction proceeded with anti-geometry and involved the transfer of the 4-*pro-R* hydrogen atom of NADPH to the 6'β position of aristeromycin as shown in Figure 1.7.

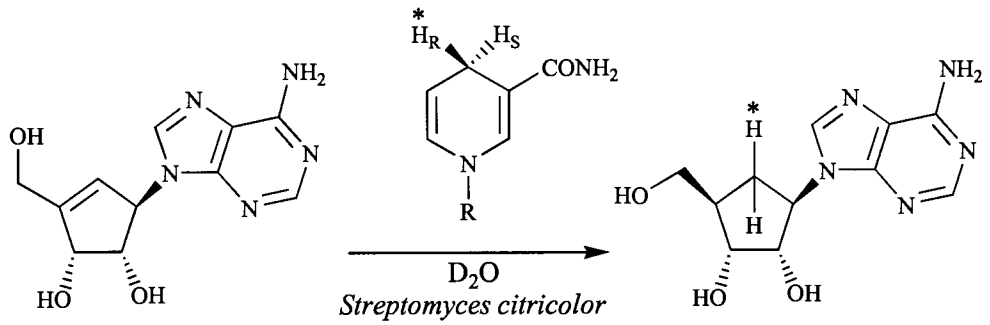
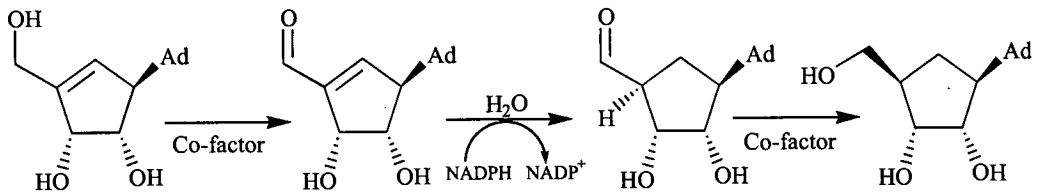


Figure 1.7 Conversion of neplanocin A to aristeromycin using a cell-free extract of *Streptomyces citricolor*

This result, which is consistent with those described above, suggested that the mechanism of conversion of neplanocin A **5** to aristeromycin **4** may involve initial oxidation of the C-5 hydroxymethyl group of neplanocin A **5** to the corresponding unsaturated aldehyde **97**, followed by conjugate reduction to the aldehyde **98**, and then reduction of the aldehyde **98** back to the alcohol to yield aristeromycin **4** as outlined in Figure 1.8.



5 **97** **98** **4**

Figure 1.8 Proposed mechanism for the reduction of neplanocin A to aristeromycin

Supporting evidence for the operation of the mechanism shown in Figure 1.8 was obtained by Turner *et al.*[84] By feeding deuterium labelled U-$^2H_7$-D-glucose Turner *et al.* have been able to map out the fate of all the C-H bonds of D-glucose during conversion to the carbocyclic portion of aristeromycin as shown in Figure 1.9.

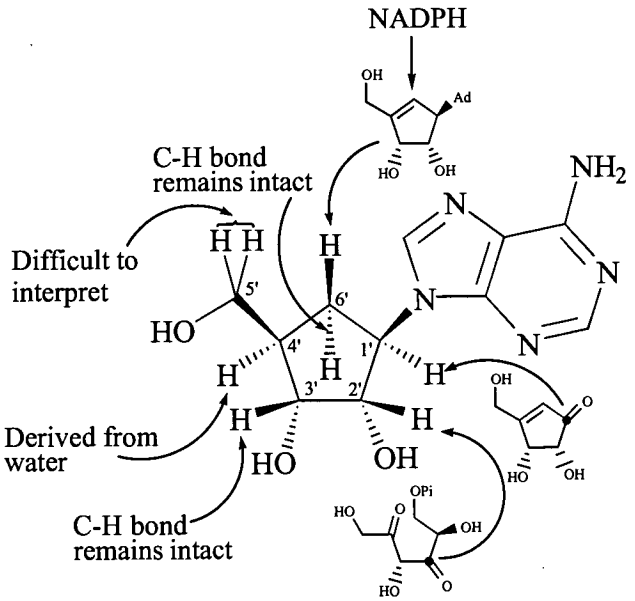


Figure 1.9 Summary of source of all C-H bonds in aristeromycin

In this experiment, the level of deuteration at C-6'*S* and C-3' showed that no loss of the deuterium label had occurred which indicated that these C-H bonds were retained intact from D-glucose. At C-1', C-2', C-5' and C-6'*R*, however, the level of deuterium incorporation was much lower which suggested that carbon-deuterium bond cleavage occurred somewhere along the pathway, followed by partial reintroduction of deuterium *via* NAD^2H. The observed partial deuterium incorporation at C-6'*R* and complete wash-out at C-5' agreed with previous experiments reported by Parry[87] and supported the proposed mechanism for the reduction of neplanocin A to aristeromycin shown in Figure 1.8. Furthermore, at C-1' and C-2' the observed low level of deuterium incorporation was consistent with the participation of the biosynthetic intermediates **82a** and **93**, respectively. No incorporation of deuterium was observed at the C-4' position which suggested that this hydrogen was derived from water.

(e) Conclusions and future experiments

Consideration of the results outlined above has led to the proposed pathway shown in Figure 1.2 for the biosynthesis of aristeromycin and neplanocin A by *Streptomyces citricolor.*

Although the core of the biosynthetic pathway is known, many issues remain to be resolved concerning the exact detail of the biosynthesis, in particular:

i. the identity of the intermediates between D-glucose **81** and the cyclopentenone **82a**.
ii. the exact nature of the intermediates **83b**, **84a** and **84b**, and in particular the exact identity of the precursor that undergoes the addition of the adenine base.
iii. the mechanism of reduction of neplanocin A to aristeromycin.

1.4 Aims

The aim of this project was to investigate two aspects of the biosynthesis, namely:

i. the mechanism of conversion of D-glucose **81** to the cyclopentenone **82a**, by identification of the first formed carbocyclic intermediate on the biosynthetic pathway.

ii. the transformation of tetrol **83a** to neplanocin A **5** *i.e.* the incorporation of the purine base, by identification of the phosphorylated intermediates prior to neplanocin A.

2 Investigations into the Conversion of D-Glucose to the Carbocyclic Ring – Identification of the First Formed Carbocyclic Intermediate

Of considerable interest in the biosynthetic pathway is the nature of the intermediates between D-glucose **81** and the cyclopentenone **82a**. By analogy with the biosynthesis of shikimic acid it is possible to suggest the route from D-glucose **81** to the cyclopentenone **82a** shown in Figure 1.4, in which the key step is the intramolecular aldol cyclisation of enol **94** to keto-tetrol **95**. From this pathway it can be speculated that the keto-tetrol **95** provides the crossover point from acyclic to cyclic intermediates and therefore the synthesis of this novel compound became the initial goal of the project.

2.1 Synthesis of the Proposed First Carbocyclic Intermediate Using a Starting Material from the Chiral Pool

2.1.1 Retrosynthetic analysis

(a) The Fujimoto-Belleau reaction

The Fujimoto-Belleau reaction, first described in 1951, involves the reaction of a nucleophile *e.g.* a Grignard reagent with an exocyclic γ-enol-lactone to give a β-hydroxycyclohexanone derivative.[88] The reaction proceeds by initial attack of the nucleophile at the carbonyl centre followed by ring opening to give an enolate at one terminus and a carbonyl at the other. Intramolecular aldol cyclisation then affords the β-hydroxycyclohexanone as shown in Figure 2.1.

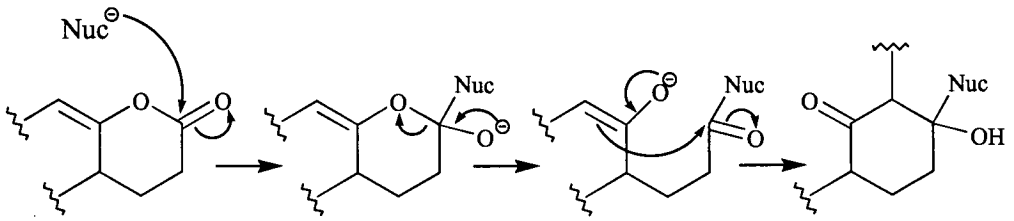
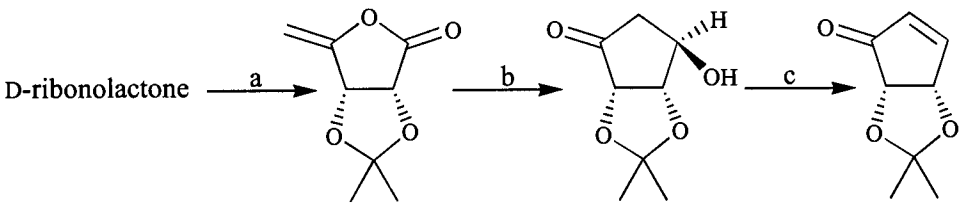


Figure 2.1 Mechanism of the Fujimoto-Belleau reaction

The Fujimoto-Belleau reaction to produce cyclohexanone derivatives has been well documented due to its common use in the total synthesis of steroids.[88] By comparison, the corresponding reaction to produce five-membered rings is relatively rare. However, Bélanger and Prasit[89] have reported the use of this methodology to prepare enantiomerically pure cyclopentanones. The Fujimoto-Belleau reaction on the enol-lactone ***ent*-99**, derived from D-ribonolactone, gave the β-hydroxy-cyclopentanone **100** which upon dehydration afforded the synthetically useful cyclopentenone **61** as outlined in Scheme 2.1.



***ent*-99** **100** **61**

Scheme 2.1 *Reagents and conditions:* a. 4 steps, 67%; b. i. $LiAlH(t\text{-}BuO)_3$, THF, 0°C; ii. aq. NH_4Cl; c. MsCl, pyridine, CH_2Cl_2.

This transformation provides a useful synthetic route to a carbocyclic nucleus from a carbohydrate precursor.

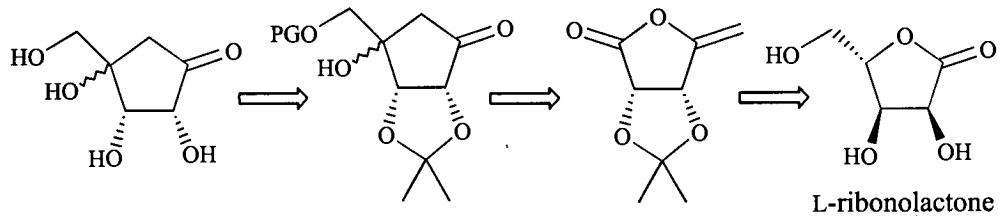
(b) Retrosynthetic plan

The desired keto-tetrol **95** is structurally related to the β-hydroxycyclopentanone **100**. By analogy to the synthesis of compound **100**, it was envisioned that the keto-tetrol **95** could be prepared according to the retrosynthetic plan shown in Scheme 2.2, in which the key step is the Fujimoto-Belleau reaction on the enol-lactone **99** to yield the protected derivative **101**.

A potential problem in the synthesis of the keto-tetrol **95** was the expected tendency for the product to undergo β-elimination of water to give the enone **82a**.

The initial work in this area was carried out by I. V. J. Archer, a post-doctoral research assistant within the group. Archer successfully developed routes to intermediates for the preparation of the keto-tetrol **95** and its enantiomer ***ent*-95** using L-ribose and D-ribonolactone respectively as starting materials. Therefore, the

initial aim was to repeat this chemistry and scale-up the steps already developed to obtain sufficient quantities of material for use in further studies.



95 **101** **99** **102**

Scheme 2.2

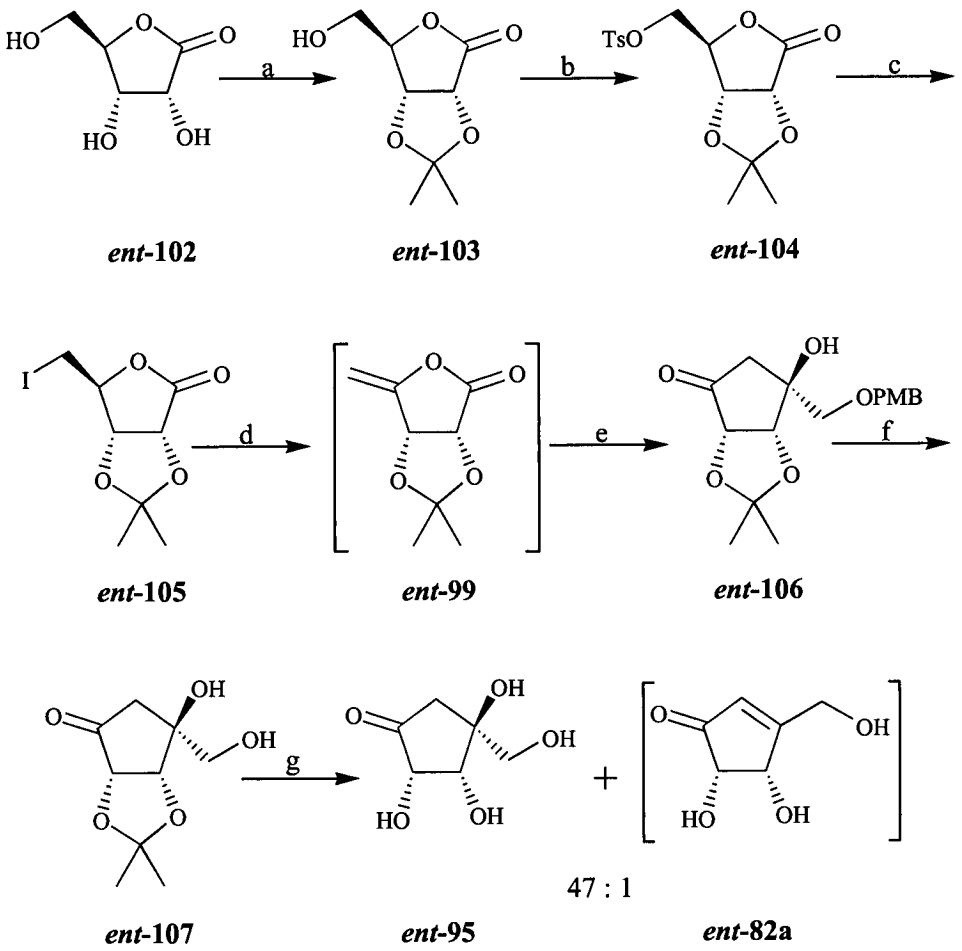
2.1.2 Synthesis of *ent*-keto-tetrol *ent*-95

In order to establish the viability of the proposed synthetic route, the synthesis of the *ent*-keto-tetrol ***ent*-95** was attempted first using the readily available D-ribonolactone ***ent*-102** as the starting material, rather than the expensive L-ribonolactone **102**. The *ent*-keto-tetrol ***ent*-95** has the incorrect absolute stereochemistry for incorporation into neplanocin A and aristeromycin. However, it was felt that it would be a useful substrate for comparative feeding studies alongside keto-tetrol **95**. These feeding studies are discussed in Section 2.4.

The *ent*-keto-tetrol, (2*S*,3*R*,4*S*)-4-hydroxymethyl-2,3,4-trihydroxy-cyclopentan-1-one ***ent*-95** was prepared from D-ribonolactone ***ent*-102** as shown in Scheme 2.3.

D-ribonolactone ***ent*-102** was firstly protected as its 2,3-acetonide derivative ***ent*-103**. The iodide ***ent*-105** was then formed in 70% overall yield by tosylation of the alcohol and then displacement of the tosylate group of ***ent*-104** using sodium iodide in refluxing acetone. These reactions were performed successfully on a large scale starting from 10 g of D-ribonolactone ***ent*-102** to give 13 g of the iodide ***ent*-105**.

Using methodology based loosely on that of Bélanger and Prasit,[89] elimination of HI from iodide ***ent*-105** with DBU followed by filtration by canular gave a solution of the enol-lactone ***ent*-99** in anhydrous THF, which was used in the next step without further isolation.



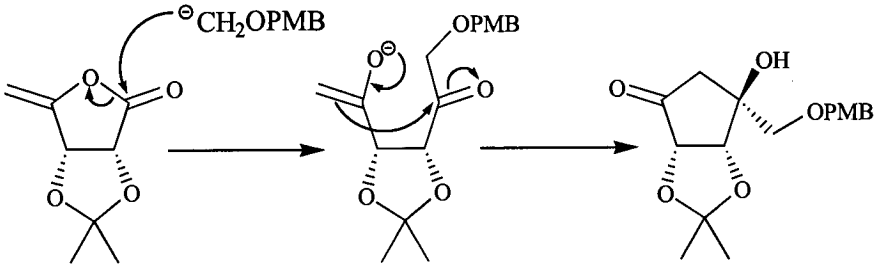
***ent*-102** ***ent*-103** ***ent*-104**

***ent*-105** ***ent*-99** ***ent*-106**

***ent*-107** ***ent*-95** ***ent*-82a**

Scheme 2.3 *Reagents and conditions:* a. acetone, PTSA, 99%; b. TsCl, pyridine, $CHCl_3$, 80%; c. NaI, acetone, reflux, 85%; d. DBU, THF; e. i. *n*-BuLi, Bu_3SnCH_2OPMB **108**, THF, -78°C; ii. aq. NH_4Cl, 35%; f. H_2, Pd/C (10%), THF, 68%; g. TFA:CH_2Cl_2 (1:4), 26%.

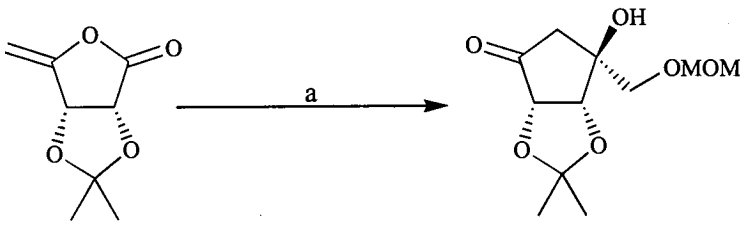
[(*p*-Methoxybenzyloxy)methyl]tri-*n*-butylstannane **108**, the synthesis of which is described in Section 3.2.2, was transmetallated with *n*-BuLi at low temperature to afford the alkyl lithium species to which the enol-lactone ***ent*-99** solution was added *via* canular. Work up by quenching at -78°C with saturated aqueous ammonium chloride solution gave the desired β-hydroxycyclopentanone ***ent*-106** as a single diastereomer in 35% yield as illustrated in Scheme 2.4.



***ent*-99** ***ent*-106**

Scheme 2.4

Confirmation of the stereochemistry of the diastereomer ***ent*-106** was obtained from preliminary studies carried out by Archer. When developing the synthetic route to the keto-tetrol, initially [(methoxymethoxy)methyl]tri-*n*-butylstannane **110** was used in the Fujimoto-Belleau reaction to give the MOM-protected β-hydroxycyclopentanone ***ent*-109** in 52% yield as shown in Scheme 2.5.



***ent*-99** ***ent*-109**

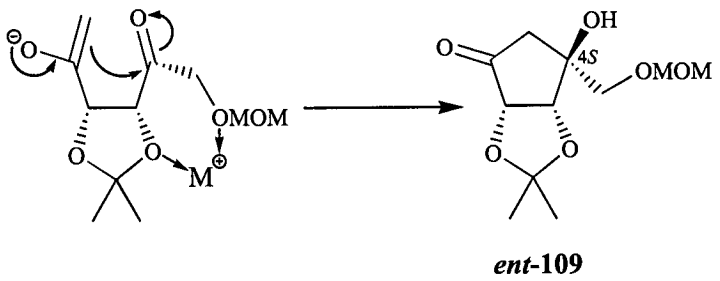
Scheme 2.5 *Reagents and conditions:* a. i. $Bu_3SnCH_2OCH_2OCH_3$ **110**, *n*-BuLi, THF, -78°C; ii. aq. NH_4Cl, 52%.

Purification of compound ***ent*-109** by column chromatography gave a crystalline solid whose relative stereochemistry was determined by X-ray crystallography. By analogy with this result the absolute stereochemistry of the PMB-protected compound ***ent*-106** was assigned.

The reason for the formation of the 4*S* diastereomer ***ent*-109** in preference to the 4*R* diastereomer was unclear. However a possible explanation for this observation is illustrated in Figure 2.2.

Chelation of the metal cation occurs between the electron rich oxygen atom of the MOM ether and the oxygen atom of the acetonide group. Therefore the CH_2OMOM

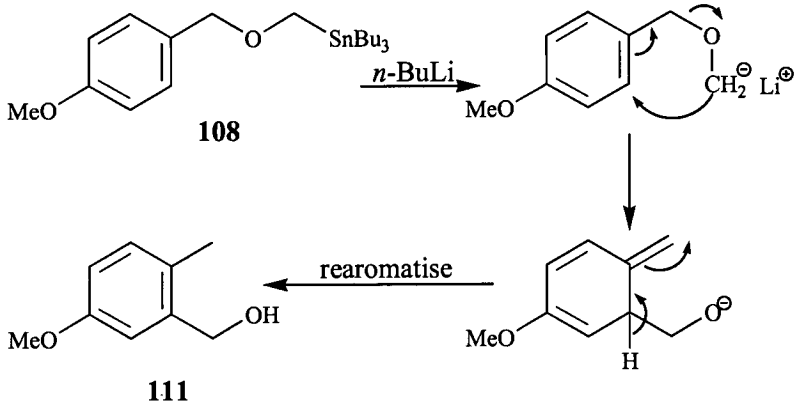
and the acetonide group end up on the same, more hindered face which results in formation of the 4*S* diastereomer ***ent*-109**.



***ent*-109**

Figure 2.2 Proposed mechanism for the preferential formation of the 4*S* diastereomer of the β-hydroxycyclopentanone

The cyclopentanone ***ent*-106** was typically isolated in yields in the range of 25% from iodide ***ent*-105**. One reason for this low yield is possibly due to the fact that the stannane **108**, when lithiated, can undergo an intramolecular Wittig rearrangement as shown in Scheme 2.6, to give compound **111** which was isolated from the reaction in 22% yield.



Scheme 2.6

It was thought that the formation of this by-product could be reduced in a number of ways. Firstly, by keeping the temperature of all solutions at -78°C. Secondly, by stirring the lithiated stannane solution for no more than five minutes and finally, by the very quick addition of the cooled enol-lactone solution to the lithiated stannane solution. By taking these factors into account the yield of β-

hydroxycyclopentanone ***ent*-106** was increased to 35%, which although not a vast increase did make a significant difference to the amount of product obtained.

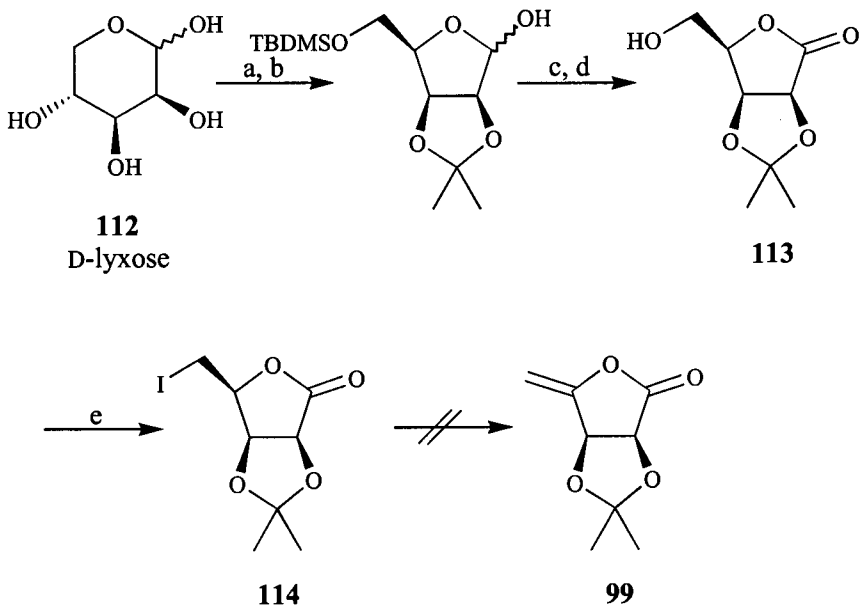
Archer investigated a number of different methods for the deprotection of the *p*-methoxybenzyl group from the β-hydroxycyclopentanone ***ent*-106**, including the use of DDQ and cerium(IV) ammonium nitrate. However, application of these methods resulted in the elimination of water from compound ***ent*-106** to give the conjugated α,β-unsaturated ketone. Removal of the *p*-methoxybenzyl group was ultimately achieved under mild conditions by catalytic hydrogenation to give the acetonide-protected diol ***ent*-107** in 68% yield. Treatment of the protected diol ***ent*-107** with trifluoroacetic acid in dichloromethane gave the desired keto-tetrol ***ent*-95** in 26% yield. However, due to the tendency of this compound to undergo β-elimination of water the keto-tetrol ***ent*-95** was contaminated with minor traces of the eliminated compound enone ***ent*-82a** (approximately 47:1 mixture of ***ent*-95**:***ent*-82a**, determined by 250 MHz ^{1}H NMR analysis) as shown in Scheme 2.3. The optimum conditions for the TFA deprotection reaction were stirring the protected diol ***ent*-107** in a 1:4 mixture of trifluoroacetic acid:dichloromethane for three minutes before quickly concentrating the solution and then purification by column chromatography. In this case although the yield of desired product obtained was poor the product was contaminated with only a trace of the eliminated compound.

It also appeared that elimination of the β-hydroxycyclopentanone type compounds occurred during their purification due to the acidity of the silica used in column chromatography. In order to reduce the acidity of the silica, the column was washed before use with the desired eluent containing a small amount of triethylamine (2 ml in 500 ml of eluent). However, this appeared to have a detrimental effect and resulted in further elimination taking place.

2.1.3 Synthesis of keto-tetrol 95

(a) Attempted synthesis from D-lyxose

Having established a viable synthetic route to the enantiomer of the keto-tetrol ***ent*-95**, Archer attempted to prepare the desired keto-tetrol **95** from D-lyxonolactone, for which syntheses have been reported in the literature.[90,91] However, the synthesis of D-lyxonolactone proved problematic and even with D-lyxonolactone in hand it was not possible to selectively protect the 2,3 positions by acetonide formation due to the preferential formation of the 3,5-acetonide – despite literature reports to the contrary.[92] Archer did however achieve the synthesis of 2,3-O-isopropylidene-D-lyxonolactone **113** from D-lyxose **112**, and using the same conditions as previously described D-lyxonolactone **113** was converted to the corresponding iodide **114** (Scheme 2.7).



112
D-lyxose

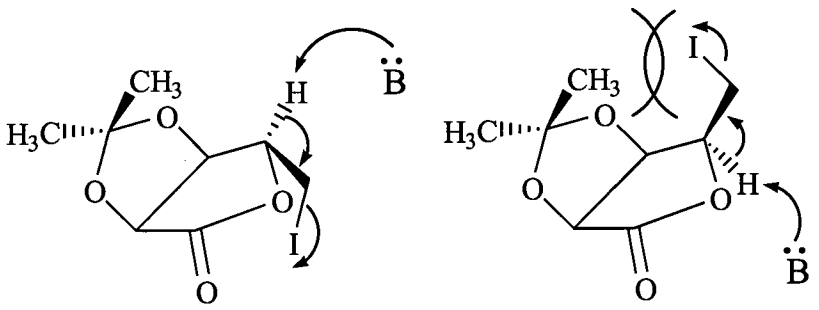
113

114

99

Scheme 2.7 *Reagents and conditions:* a. acetone, H_2SO_4, quant.; b. TBDMSCl, imidazole, 80%; c. $KMnO_4$, 79%; d. HF, 73%; e. i. TsCl, pyridine; ii. NaI, acetone, 70%.

Unfortunately, elimination of HI from the iodide **114** was not possible, probably due to its inability to adopt a conformation in which the iodine and hydrogen atoms to be removed are anti-periplanar to each other as shown in Figure 2.3.

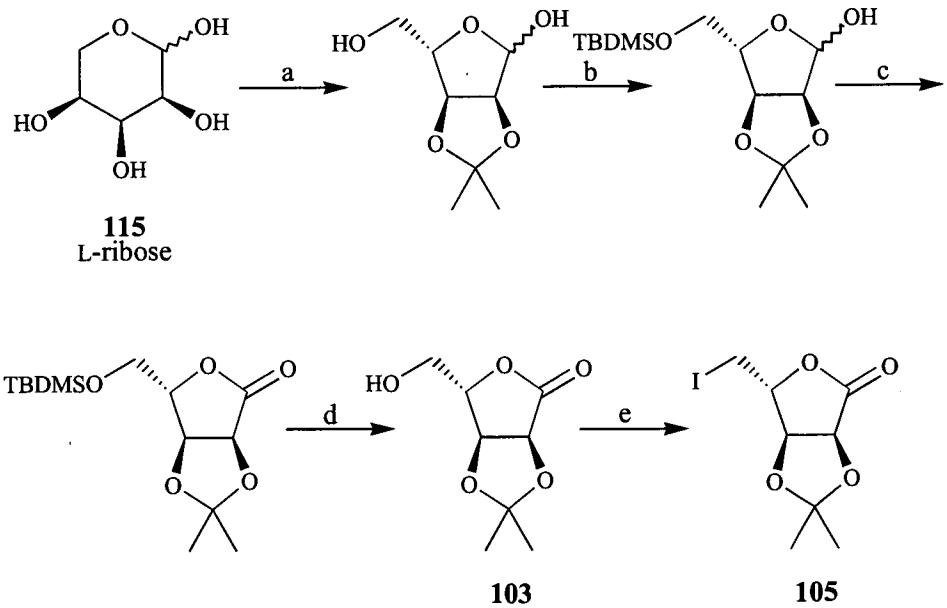


D-ribose series D-lyxose series

Figure 2.3 Mechanism of HI elimination

(b) Synthesis from L-ribose

In view of the successful synthesis of ***ent*-95** using D-ribonolactone as the starting material it was concluded that the preparation of the desired keto-tetrol **95** could only be achieved by the use of L-ribose as the starting material. L-ribose, although very expensive, is commercially available. Using the synthetic route shown in Scheme 2.8, Archer was able to convert L-ribose **115** to the key iodide **105**.

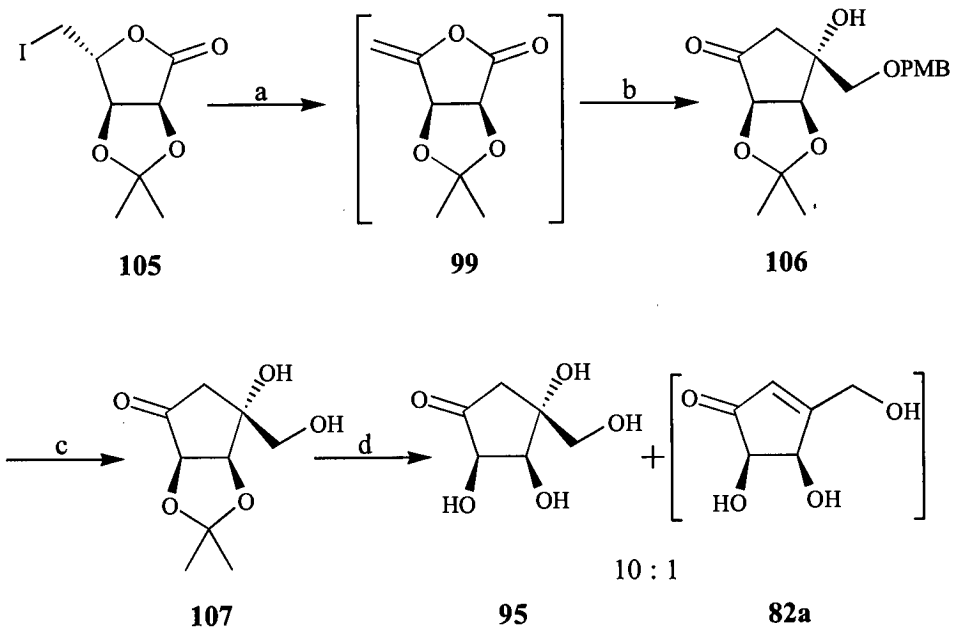


115
L-ribose

103 **105**

Scheme 2.8 *Reagents and conditions:* a. acetone, H_2SO_4, quant.; b. TBDMSCl, imidazole, 80%; c. $KMnO_4$, acetone, 78%; d. 48-51% HF, MeCN, THF, 78%; e. i. TsCl, pyridine; ii. NaI, acetone, 70%.

The iodide **105** prepared by Archer was then used to prepare the desired keto-tetrol **95** using the previously developed methodology as illustrated in Scheme 2.9.



Scheme 2.9 *Reagents and conditions:* a. DBU, THF; b. i. *n*-BuLi, Bu_3SnCH_2OPMB **108**, THF, -78°C; ii. aq. NH_4Cl, 55%; c. H_2, Pd/C (10%), THF, 57%; d. TFA:CH_2Cl_2 (1:4), 40%.

Elimination of HI from iodide **105** was achieved by treatment with DBU followed by filtration *via* canular to give a solution of the enol-lactone **99** in THF. [(*p*-Methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** was treated with *n*-BuLi and the enol-lactone **99** solution was added quickly to this *via* canular to give after work up the protected β-hydroxycyclopentanone **106** in 55% yield. The yield obtained (55%) was significantly better than for the enantiomeric D-series, which was thought to be due to the very rapid addition of the enol-lactone solution to the lithiated stannane solution. Verification of the stereochemistry of the newly formed stereocentre in β-hydroxycyclopentanone **106** was obtained by ^1H NMR nOe experiments, the results of which are discussed in Section 2.2.2 and illustrated in Figure 2.4.

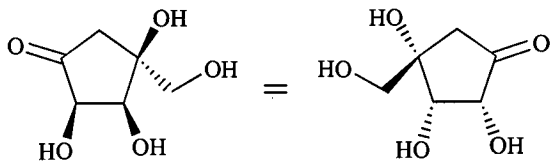
The PMB-protecting group was removed from compound **106** by catalytic hydrogenation to give the protected diol **107** in 57% yield.

Treatment of the diol **107** with trifluoroacetic acid gave the desired keto-tetrol **95** in 40% yield. As before the keto-tetrol **95** was contaminated with minor traces of

the enone **82a** (approximately 10:1 mixture of **95**:**82a**, determined by 600 MHz ^{1}H NMR analysis).

2.2 Synthesis of the Proposed First Carbocyclic Intermediate by Enzymatic Desymmetrisation

At this point synthetic routes had been established for the preparation of the keto-tetrol **95** and its enantiomer ***ent*-95** from L-ribose and D-ribonolactone, respectively. The availability of ***ent*-95** was important for comparative feeding studies alongside keto-tetrol **95**, as discussed in Section 2.4. It also became clear that the C-4 diastereomer of the keto-tetrol **95**, namely (2*R*,3*S*,4*S*)-4-hydroxymethyl-2,3,4-trihydroxy-cyclopentan-1-one **116** may be an intermediate on the biosynthetic pathway. The synthesis of this compound therefore became the next goal of the project.



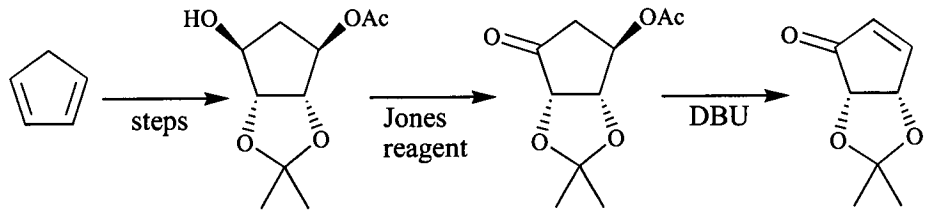
116

2.2.1 Retrosynthetic analysis

In the previous syntheses of the keto-tetrol **95**, and its enantiomer ***ent*-95**, the stereochemistry at the 4-position was established by the Fujimoto-Belleau reaction. It had been hoped that the Fujimoto-Belleau reaction would give a mixture of separable diastereomers to provide a convenient route to both diastereomers of the keto-tetrol. However, the reaction produced a single diastereomer and therefore it was not possible to prepare the keto-tetrol **116** from a carbohydrate by this procedure.

Studies by Archer towards the synthesis of the synthetically useful cyclopentenone **61** resulted in preparation of the novel β-acetoxycyclopentanone

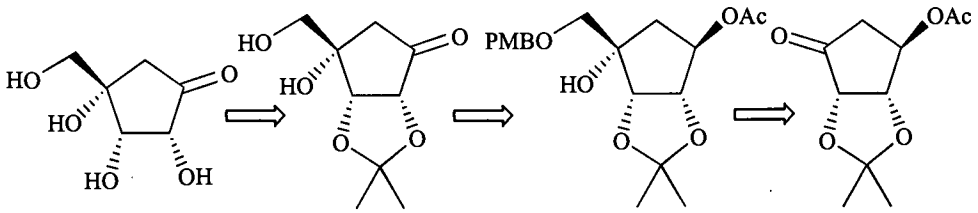
118. Using a revised procedure to that described by Johnson and Penning,[93] Archer converted cyclopentadiene **36** to the monoacetate **117** as outlined in Scheme 2.10.



36 **117** **118** **61**

Scheme 2.10

By conversion of the monoacetate **117** to the cyclopentenone **61** following the procedure reported by Johnson and Penning,[93] Archer isolated the novel β-acetoxycyclopentanone **118** in 85% yield. It was thought that this β-acetoxy-cyclopentanone **118** could be used to prepare the desired diastereomer of the keto-tetrol **116** according to the retrosynthetic plan shown in Scheme 2.11, in which the key step to introduce the stereochemistry at the 4-position is the addition of the lithiated stannane **108** to the carbonyl group of the β-acetoxycyclopentanone **118**.



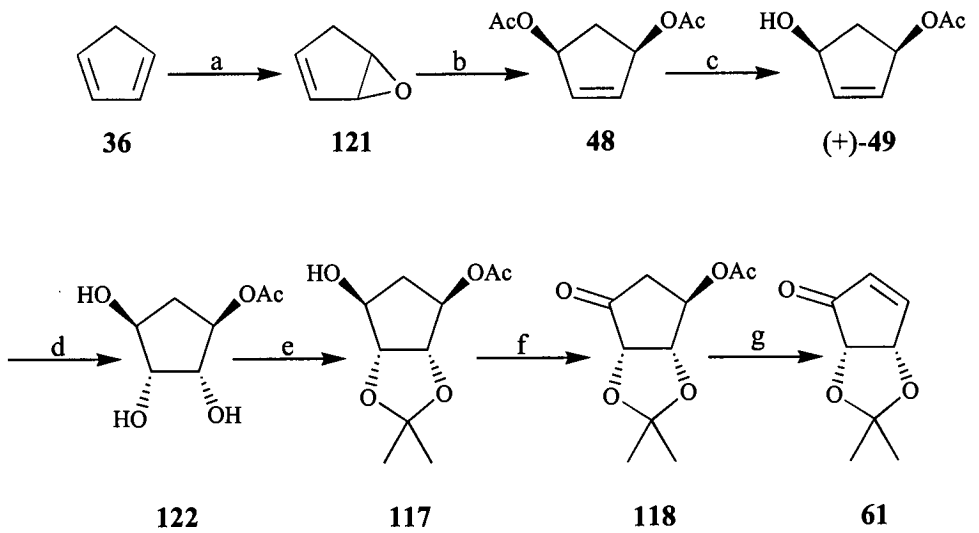
116 **119** **120** **118**

Scheme 2.11

I. Jackson, a project student within the group investigated the synthesis of the keto-tetrol **116** using this methodology. However due to time constraints he was unable to complete the synthesis of the desired keto-tetrol **116**. Therefore the initial task was to repeat and scale-up the previously developed steps and then to complete the synthesis of the keto-tetrol **116**.

2.2.2 Synthesis of keto-tetrol 116

The β-acetoxycyclopentanone **118** was prepared from cyclopentadiene **36** as shown in Scheme 2.12.



Scheme 2.12 *Reagents and conditions:* a. CH_3CO_3H, NaOAc, Na_2CO_3, CH_2Cl_2, 24%; b. $Pd(PPh_3)_4$, Ac_2O, THF, 59%; c. CAL-B (Novo SP-435), phosphate buffer (pH 8.0), 85%; d. OsO_4, NMO, THF, acetone, H_2O, 88%; e. acetone, PTSA, 93%; f. Jones reagent, acetone, 60%; g. DBU, THF, 91%.

Monoepoxidation of cyclopentadiene **36** was performed by treatment of freshly distilled cyclopentadiene **36** with buffered peracetic acid solution following the procedure described by Knapp,[94] to give the racemic cyclopentadiene monoepoxide **121** in 24% yield based on cyclopentadiene. Although the yield obtained was poor, the starting materials were very cheap and the reaction was relatively easy to perform on a large scale starting from 96 g of cyclopentadiene.

Following the literature procedure,[51] treatment of cyclopentadiene monoepoxide **121** with acetic anhydride in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0) gave the *cis*-diacetate **48** in 59% yield.

Desymmetrisation of the *meso*-diacetate **48** was achieved by enzymatic hydrolysis using *Candida antarctica* lipase B in phosphate buffer to afford the monoacetate (+)-**49** in 85% yield and >99% e.e. determined by chiral gas chromatography. *Candida antarctica* lipase B (CAL-B) is supplied immobilised as an acrylic supported biocatalyst (Novo SP-435). The use of this immobilised enzyme is particularly

advantageous as the enzyme can be recovered from the reaction and reused. The hydrolysis of diacetate **48** to provide monoacetate **49** in high enantiomeric purity has also been carried out by the use of porcine pancreatic lipase (PPL),[95] *Pseudomonas fluorescens* lipase (PCL)[52] and electric eel acetylcholinesterase (EEACE).[96] Archer initially investigated the use of EEACE for the desymmetrisation reaction. However due to inhibition of this enzyme by AcOH production, which prevented the reaction from going to completion, a poorer yield and e.e. were obtained. EEACE is also expensive (£130/20000 units required for one 10 g reaction).

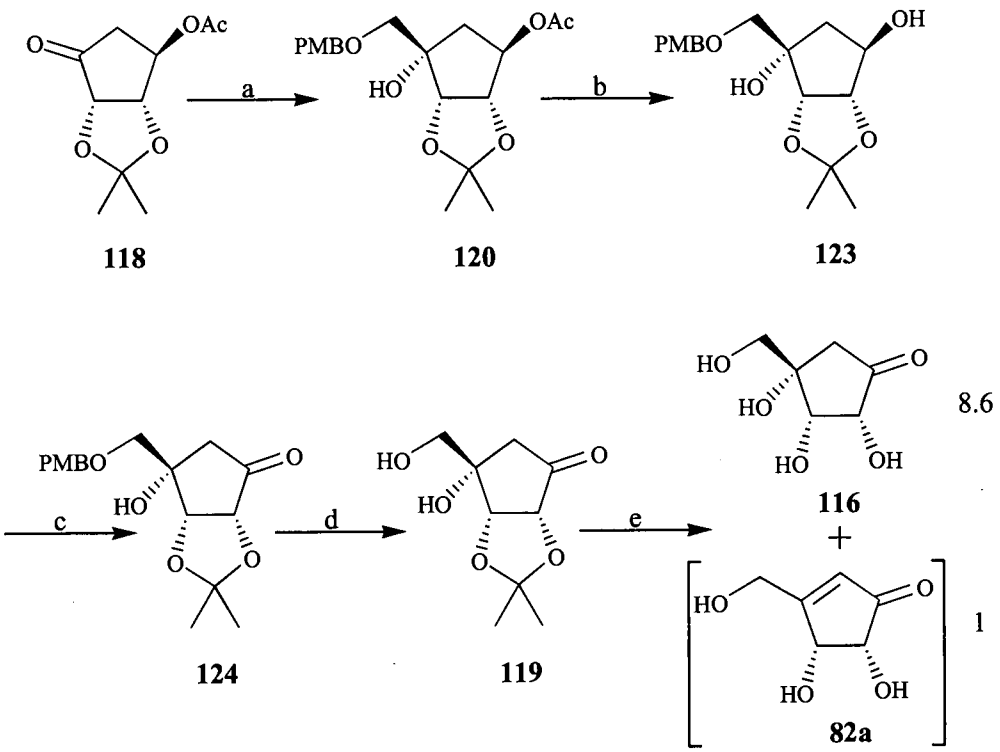
cis-Dihydroxylation of the monoacetate (+)-**49** from the less hindered face, using a catalytic amount of osmium tetroxide in the presence of the oxidant 4-methyl-morpholine *N*-oxide afforded the diol **122** in 88% yield. Some problems were encountered with the aqueous work-up of this reaction. Extraction of the desired diol **122** from the aqueous portion was very difficult and resulted in low overall yields. To overcome this problem the crude reaction mixture was concentrated and then directly purified by column chromatography without prior work-up. This resulted in a high yield of the diol **122** being obtained as an oily white solid. The diol **122** was then protected as its 2,3-acetonide derivative **117** by treatment with acetone and *p*-toluenesulfonic acid.

The final oxidation step for the synthesis followed the same procedure as that carried out by Johnson[93] but did not yield the same product. In our hands, treatment of the acetonide protected diol **117** with Jones reagent gave the β-acetoxy-cyclopentanone **118** in 60% yield as a white solid after purification by recrystallisation using diethyl ether. In Johnson's work oxidation of the acetonide protected diol **117** with Jones reagent afforded the α,β-unsaturated ketone **61**. It was thought that the β-acetoxycyclopentanone **118** was not isolated in Johnson's case as the product of the oxidation was purified by column chromatography, which due to the acidity of the silica causes elimination of the β-acetoxycyclopentanone **118** to form the cyclopentenone **61**. In our work, purification of the β-acetoxy-cyclopentanone **118** by column chromatography did indeed result in elimination taking place to give a mixture of the β-acetoxycyclopentanone **118** and the cyclopentenone **61**, therefore purification of the β-acetoxycyclopentanone **118** by this procedure was not possible. As the β-acetoxycyclopentanone **118** was a

crystalline solid the X-ray crystal structure was obtained, which confirmed the absolute stereochemistry of the compound. The crystal structure of β-acetoxycyclopentanone **118** is given in Appendix 1.

The cyclopentenone **61**, if required, can easily be obtained by elimination of AcOH from the β-acetoxycyclopentanone **118** using DBU giving the desired enone **61** in 91% yield.

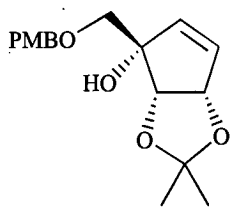
The β-acetoxycyclopentanone **118** was then converted to the desired keto-tetrol **116** following the synthetic route shown in Scheme 2.13.



Scheme 2.13 *Reagents and conditions:* a. Bu_3SnCH_2OPMB **108**, *n*-BuLi, THF, 19%; b. NH_3 sat. MeOH, 92%; c. IBX, DMSO, 88%; d. H_2, Pd(C), THF, 36%; e. TFA:CH_2Cl_2 (1:4), 21%.

Treatment of [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** with *n*-BuLi generated the corresponding alkyl lithium species which underwent addition to the carbonyl group of the β-acetoxycyclopentanone **118** to afford the PMB-protected acetate **120** in poor yield ranging from 7% to 19%. The cyclopentenone **61** (50%) and the PMB-protected alcohol **125** (7%) were also isolated from the reaction. The

latter was presumably obtained by the addition of the hydroxymethyl anion equivalent to the cyclopentenone **61**.



125

This result suggests that the majority of the starting β-acetoxycyclopentanone **118** is eliminating under the reaction conditions to give the cyclopentenone **61**. Attempts to reduce the amount of enone **61** and increase the amount of acetate **120** formed by the use of different temperatures and modes of addition were unsuccessful. Replacement of the acetate group of the β-acetoxycyclopentanone **118** with a more robust protecting group may reduce the amount of elimination to the cyclopentenone **61**. However, due to time constraints, investigation of this idea was not attempted.

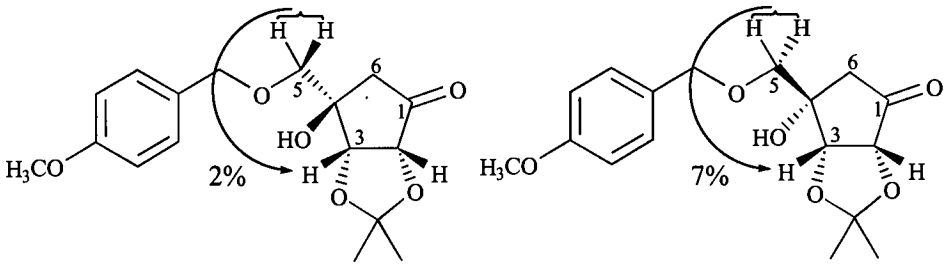
The cyclopentenone **61** and PMB-protected alcohol **125** produced as by-products can however be used to prepare the tetrol **83a** which is required as a control in the feeding experiments. The synthesis of the tetrol **83a** from these compounds is described in Section 3.2.2.

The acetate group was removed from the PMB-protected acetate **120** by the addition of ammonia saturated methanol to give the PMB-protected alcohol **123** in 92% yield, which was then oxidised using IBX in DMSO to the protected β-hydroxycyclopentanone **124** in 88% yield. The preparation of IBX is discussed in Section 3.2.1.

^{1}H NMR nOe experiments were performed on both diastereomers of the protected β-hydroxycyclopentanone **106** and **124** to verify the stereochemistry at the 4-position. The results are illustrated in Figure 2.4.

The nOe enhancements, which result from through space interactions, suggest that the *p*-methoxybenzyloxymethyl group in diastereomer **124** is *cis* to the CH-3 proton

due to the large percentage enhancement (7%) observed compared to that in diastereomer **106** (2%).



106 **124**

Figure 2.4 Results of nOe experiments

The β-hydroxycyclopentanone **124** was then converted to the keto-tetrol **116** *via* the same route as used for the previous syntheses. Removal of the PMB group was achieved by catalytic hydrogenation in only 36% yield, which is very poor compared to that for the previous hydrogenations. The reason for this is not known, however it may be due to the inaccessibility of the palladium catalyst to the reaction site in this diastereomer. The X-ray crystal structure of the β-hydroxycyclopentanone **119** was obtained and confirmed that this diastereomer did indeed have the *S*-configuration at the 4-position, which is in agreement with the nOe data presented in Figure 2.4. The crystal structure of β-hydroxycyclopentanone **119** is shown in Appendix 2.

Finally the acetonide group was removed by exposure of the β-hydroxycyclopentanone **119** to trifluoroacetic acid in dichloromethane to give the desired keto-tetrol **116** in 21% yield. As in the previous syntheses the keto-tetrol **116** was contaminated with traces of the enone **82a** (approximately 8.6:1 mixture of **116**:**82a**, determined by 600 MHz ^{1}H NMR analysis).

Having achieved the synthesis of the keto-tetrol **95** and its diastereomer **116** it was then necessary to establish whether these compounds lie on the biosynthetic pathway. This was done by carrying out feeding experiments using a mutant of *Streptomyces citricolor*. These feeding studies are discussed in detail in Section 2.4.

Due to the instability of the keto-tetrols, these compounds were synthesised in parallel and used immediately in the feeding studies.

2.3 Synthesis of Known Intermediates on the Biosynthetic Pathway for Use in Feeding Studies

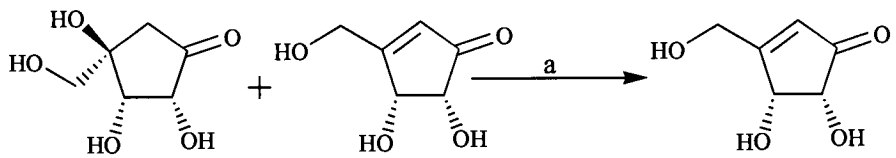
In order to carry out the feeding experiments it was necessary to use a control compound which is known to be converted by *Streptomyces citricolor* to neplanocin A **5** and aristeromycin **4**. The use of a control compound ensures that the organism is functioning efficiently and capable of converting the substrates to neplanocin A and aristeromycin. The compound which has most commonly been used in this way is the tetrol **83a**, however the enone **82a** has also been used.

2.3.1 Synthesis of tetrol 83a

The synthesis of the tetrol **83a** has been achieved from D-ribose and is described in detail in Section 3.2.

2.3.2 Synthesis of enone 82a

The enone **82a**, which is a known intermediate on the biosynthetic pathway, was prepared from the keto-tetrol **95** as shown in Scheme 2.14.



95 **82a** **82a**

Scheme 2.14 *Reagents and conditions:* a. TFA:CH_2Cl_2:CH_3OH (6:4:1), ~45%.

A mixture of the keto-tetrol **95** and enone **82a**, obtained from the trifluoroacetic acid deprotection reaction of the acetonide protected diol **107**, was stirred overnight in a solution of trifluoroacetic acid in dichloromethane and methanol (6:4:1). Concentration of the solution followed by purification by column chromatography gave the enone **82a** in approximately 45% yield.

2.4 Feeding Studies

As described in Section 1.3.1 (a), previous studies within the Turner group led to the isolation of mutant strains of *Streptomyces citricolor* that were blocked in their ability to synthesise neplanocin A **5** and aristeromycin **4**. However the production of aristeromycin could be rescued by certain combinations of mutants in which the supernatant from cultures of one mutant (secretor) were added to a culture of a second mutant (converter). Such cosynthesis experiments identified a secretor/converter pairing in which the sterile aqueous concentrate prepared from a freeze-dried culture broth of one mutant (CC914) was able to support production of neplanocin A and aristeromycin when fed to a second mutant (CC940) as illustrated in Figure 1.3. It was subsequently shown that the aristeromycin precursor from the freeze-dried supernatant was the tetrol **83a**.[82]

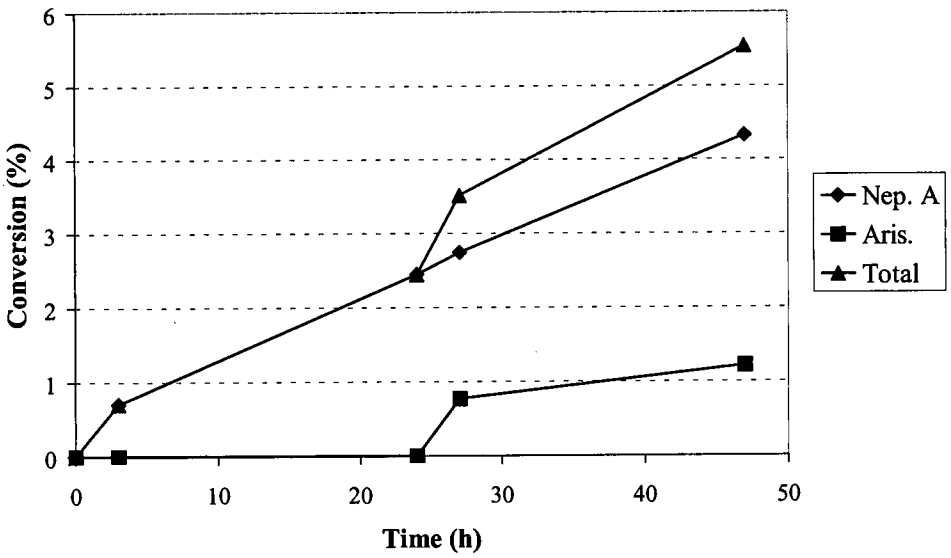
The identification of the converter mutant (CC940), which appeared to be blocked in the early steps of aristeromycin biosynthesis, allowed putative intermediates to be synthesised and fed to the mutant – the production of neplanocin A and aristeromycin suggested that the compound lies on the biosynthetic pathway. Through these studies it was established that the enone **82a** was an intermediate on the biosynthetic pathway.[83]

The aim of this work was to feed the synthesised keto-tetrol compounds to the *Streptomyces citricolor* converter mutant CC940 and if production of aristeromycin and neplanocin A was observed these compounds were deemed to lie on the biosynthetic pathway.

In this study all feeding experiments were performed using the *Streptomyces citricolor* mutant strain CC940 grown on GAM 6:6 medium supplemented with uracil. Vegetative mycelium was used to inoculate a conical flask containing the medium and after three days of growth at 30°C, 270 rpm, aliquots of the culture were transferred to several sterile conical flasks. Following addition of the substrates (to give a final concentration of 1 mg/ml) the incubation was continued at 30°C, 270 rpm for up to 7 days and the transformation was monitored by the production of neplanocin A and aristeromycin by HPLC analysis of the fermentation broth at regular intervals.

2.4.1 Tetrol feeding experiment

The CC940 mutant strain of *Streptomyces citricolor* had been stored as a lyophilised sample for a number of years. In order to establish that the mutant had been successfully revived and would efficiently convert the substrates to neplanocin A and aristeromycin, the first feeding experiment carried out used the known intermediate on the biosynthetic pathway, the tetrol **83a**. The results of this experiment are shown in Graph 2.1. A control flask is used in each feeding experiment which contains no added substrate and therefore shows no conversion to neplanocin A and aristeromycin. The plot for this control experiment is not shown on any of the graphs for the feeding experiments.



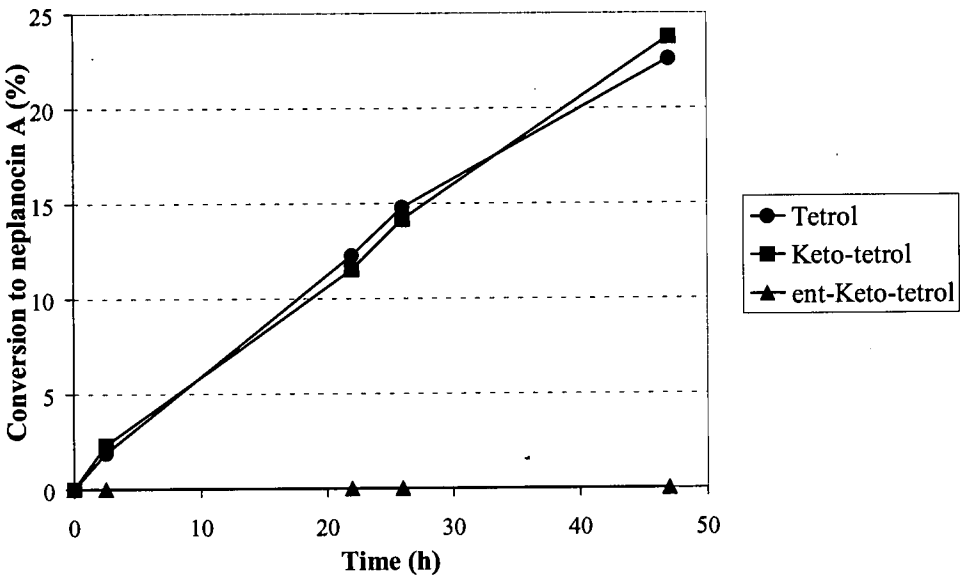
Graph 2.1 Conversion of tetrol to neplanocin A and aristeromycin

From Graph 2.1 it can be seen that contrary to previous studies within the group in which aristeromycin **4** was the major bioconversion product, the mutant CC940 bioconverts the tetrol **83a** to neplanocin A **5** as the major product. However upon prolonged incubation the amount of aristeromycin produced gradually increases. In order to be certain that neplanocin A and aristeromycin were indeed being produced, the fractions corresponding to these compounds were collected from the HPLC and subjected to mass spectrometry. Mass peaks corresponding to neplanocin A were

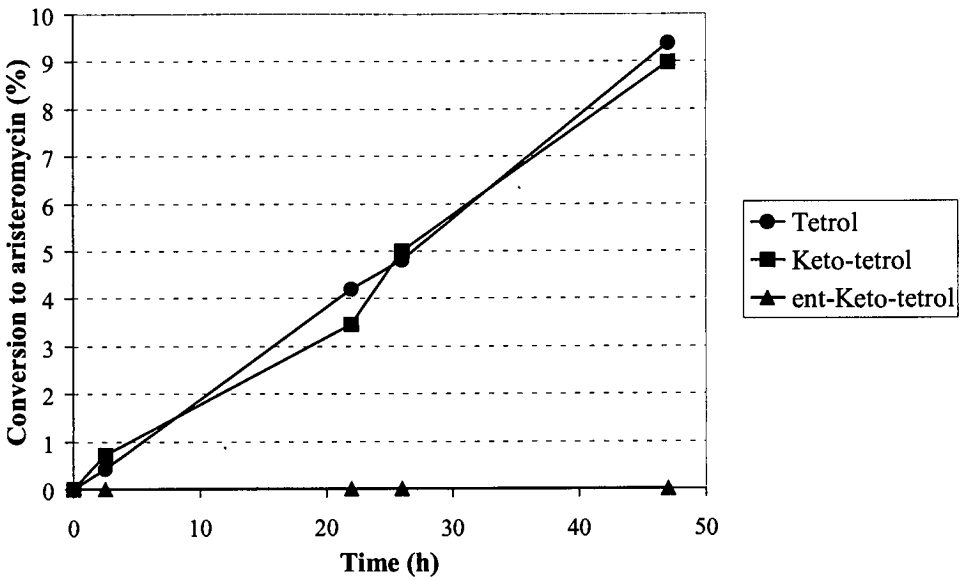
observed at *m/z* (ES+) 263.8 (MH^+, 30%) and 285.9 (M + Na, 50%). Unfortunately a mass spectrum of the aristeromycin fraction could not be obtained due to the small amount of product recovered.

2.4.2 Keto-tetrol (both enantiomers) feeding experiment

Having established that the CC940 mutant had been successfully revived and was capable of converting substrates to neplanocin A and aristeromycin, the feeding experiments using the synthesised keto-tetrol compounds were then carried out. The freshly prepared keto-tetrol **95** and its enantiomer ***ent*-95** were fed to the *Streptomyces citricolor* mutant CC940 as well as the tetrol **83a**, to ensure that the mutant was converting to neplanocin A and aristeromycin. The results of this experiment are shown in Graphs 2.2a and b.



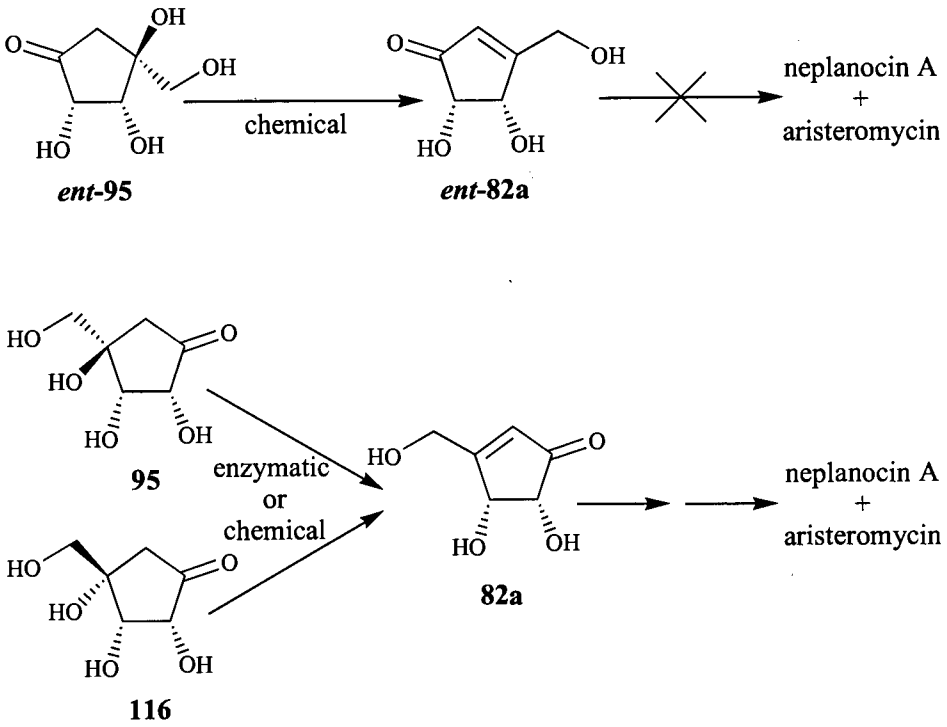
Graph 2.2a Conversion to neplanocin A



Graph 2.2b Conversion to aristeromycin

The results show that the keto-tetrol **95** is converted by *Streptomyces citricolor* to neplanocin A and aristeromycin. As expected the enantiomer ***ent*-95**, which has the incorrect absolute stereochemistry for incorporation into neplanocin A and aristeromycin, shows no conversion to neplanocin A or aristeromycin as illustrated in Scheme 2.15. As in the previous feeding experiment the major product produced is neplanocin A. As before the fractions corresponding to neplanocin A and aristeromycin were collected from the HPLC and subjected to mass spectrometry. Mass peaks corresponding to neplanocin A were observed at *m/z* (ES+) 263.6 (MH^+, 60%) and 285.9 (M+Na, 10%). Mass peaks corresponding to aristeromycin were observed at *m/z* (ES+) 265.8 (MH^+, 50%) and 287.8 (M+Na, 20%).

From the result of this experiment it was not known if the keto-tetrol **95** was converted to the enone **82a** enzymatically by *Streptomyces citricolor* or if it was simply undergoing spontaneous chemical elimination in the fermentation broth to the enone **82a** and it was subsequently this compound, which is a known intermediate on the biosynthetic pathway, that was being converted to neplanocin A and aristeromycin as shown in Scheme 2.15.

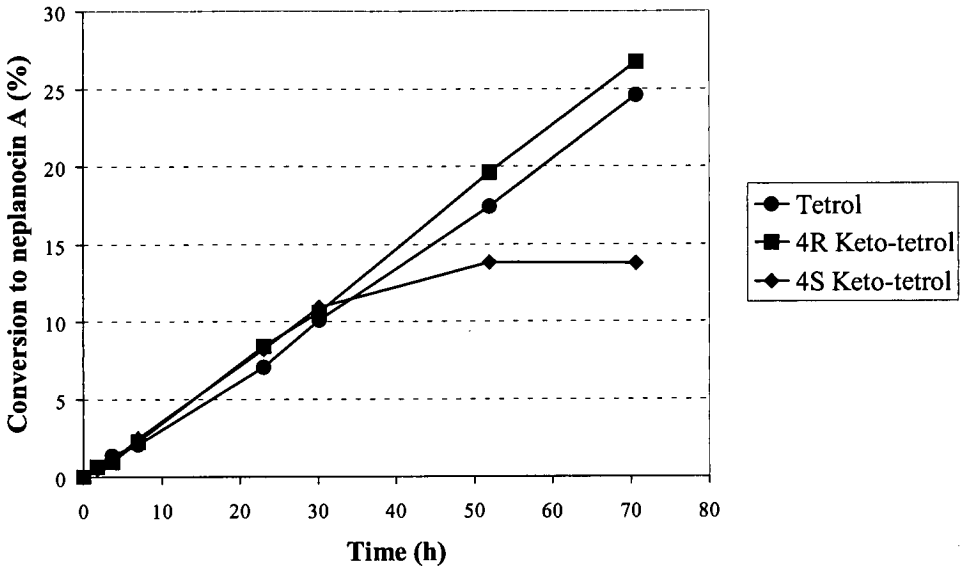


Scheme 2.15

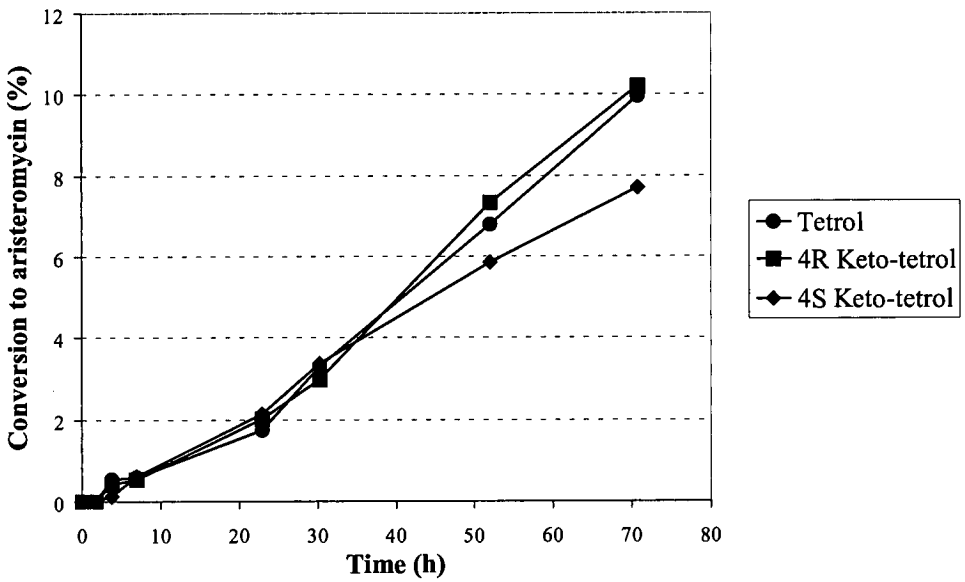
As the keto-tetrol **95** and its diastereomer **116** are both converted to the same enone **82a** (Scheme 2.15), the next experiment involved feeding both of these compounds to the *Streptomyces citricolor* mutant. If the diastereomer **116** showed no conversion to neplanocin A and aristeromycin then it could be deduced that the keto-tetrol **95** is enzymatically converted to the enone **82a** and that the keto-tetrol **95** lies on the biosynthetic pathway.

2.4.3 Keto-tetrol (both diastereomers) feeding experiment

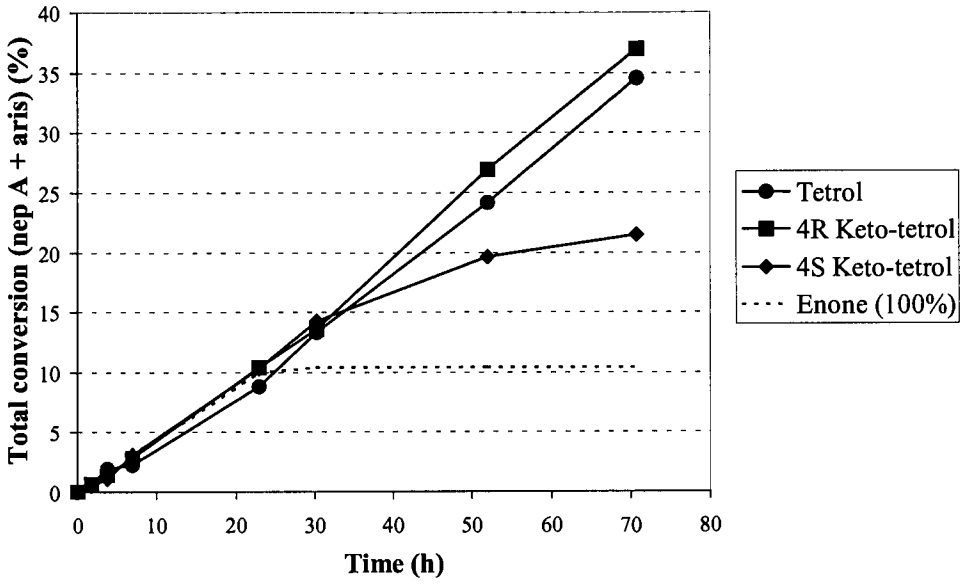
The keto-tetrol **95** and its diastereomer **116** were fed to the mutant CC940, as well as the tetrol **83a**. The results of these experiments are shown in Graphs 2.3a, b and c.



Graph 2.3a Conversion to neplanocin A



Graph 2.3b Conversion to aristeromycin



Graph 2.3c Conversion to neplanocin A + aristeromycin

From Graphs 2.3a and b, it can be seen that both the 4*R* keto-tetrol **95** and 4*S* keto-tetrol **116** are converted by *Streptomyces citricolor* to neplanocin A and aristeromycin. As the diastereomer **116** is also converted it is not possible to be sure whether the keto-tetrol compounds are enzymatically converted by *Streptomyces citricolor* or if they undergo spontaneous chemical elimination to the enone **82a**. To investigate the likelihood of the keto-tetrol compounds eliminating chemically to the enone **82a** under the conditions of the feeding study, an NMR experiment was carried out. The pH of the fermentation broth throughout the duration of the feeding experiment was approximately 5, therefore an NMR sample of the keto-tetrol **95** was made up in deuterium oxide and the pH was carefully adjusted to 5 using deuterated trifluoroacetic acid. The 200 MHz ^{1}H NMR spectrum of the sample was then obtained at regular intervals and the ratio of keto-tetrol **95**:enone **82a** was determined by comparison of the integrals. The results of these experiments are shown in Table 2.1.

	Keto-tetrol **95**	:	Enone **82a**
1 day	20.0		1
4 days	17.5		1
9 days	10.4		1
13 days	6.1		1
17 days	5.0		1
24 days	2.8		1
36 days	2.0		1
then pH of sample adjusted to 4 using deuterated trifluoroacetic acid			
4 min	1.9		1
4 h	1.9		1
1 day	1.8		1
5 days	1.5		1
then pH of sample adjusted to 3 using deuterated trifluoroacetic acid			
15 min	1.5		1
1 day	1.5		1
7 days	1.3		1
15 days	1.1		1

Table 2.1 Rate of elimination of keto-tetrol **95** to enone **82a**

These results suggest that the rate of chemical elimination of the keto-tetrol **95** to the enone **82a** would not be fast enough to have an effect on the feeding experiments, the longest of which was run for six days.

The stability of the keto-tetrol compounds was also established during the synthesis of the enone **82a** from the keto-tetrol **95** as described in Section 2.3.2. The keto-tetrol **95** was treated with an excess of trifluoroacetic acid (pH<<1) and even after stirring at room temperature overnight a small amount of the keto-tetrol **95** still remained. This result suggests that at pH 5 the chemical elimination would be very slow indeed. From these investigations it was concluded that the keto-tetrol compounds were undergoing enzymatic conversion by *Streptomyces citricolor* to the enone **82a**.

From Graph 2.3a it can be seen that conversion of the 4*S* keto-tetrol **116** to neplanocin A appears to stop after approximately forty hours whilst the conversion of the 4*R* keto-tetrol **95** and tetrol **83a** to neplanocin A continues. It was suggested that this conversion observed for the 4*S* keto-tetrol **116** may not in fact be due to

bioconversion of the keto-tetrol but instead it is the conversion of the trace amount of enone **82a** which is present as an impurity in the starting keto-tetrols. However, the total conversion (neplanocin A + aristeromycin) observed is high (approximately 21%) and the 4*S* keto-tetrol **116** is contaminated with only a small amount of the enone **82a** (10.4%). Therefore this amount of enone **82a** in the starting sample could not account for such a high percentage conversion. This is shown by the dotted line on Graph 2.3c. This line represents 100% conversion of the enone **82a**, which contaminates the 4*S* keto-tetrol **116**, to neplanocin A and aristeromycin.

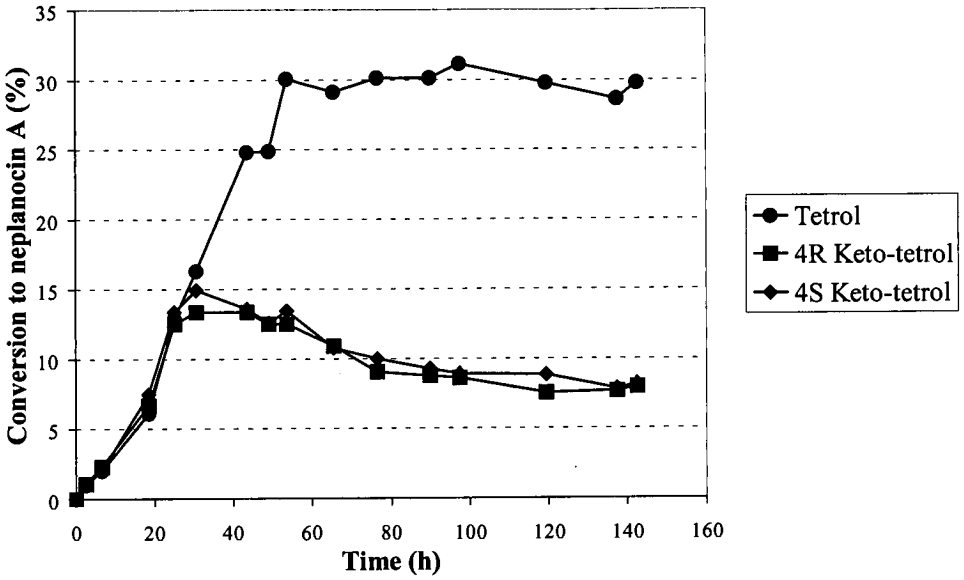
The more likely explanation for this halt in conversion is due to the small volume of fermentation broth that remains at this point in the experiment. The small volume means that the broth cannot be efficiently aerated and therefore the conversion may cease.

The feeding experiment using both diastereomers of the keto-tetrol was therefore repeated using a larger amount of substrate and hence a greater volume of medium. This allowed the feeding study to be monitored for a longer period of time.

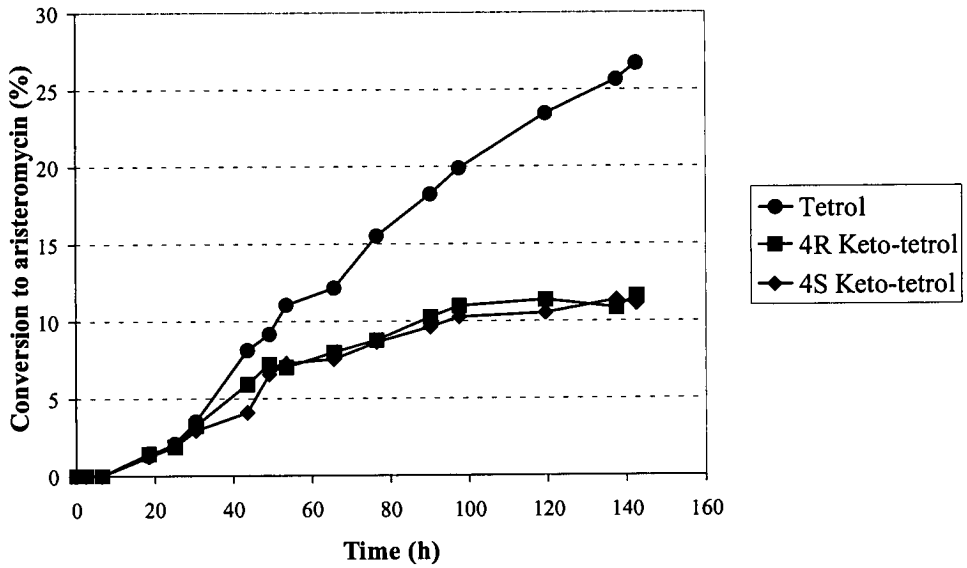
2.4.4 Keto-tetrol (both diastereomers) feeding experiment

The freshly prepared 4*R* keto-tetrol **95** and its diastereomer 4*S* keto-tetrol **116** were fed to the *Streptomyces citricolor* mutant CC940. As in the previous feeding experiments the tetrol **83a** was also fed to the mutant to ensure that it was converting the substrates to neplanocin A and aristeromycin. The results of this study are given in Graphs 2.4a, b and c.

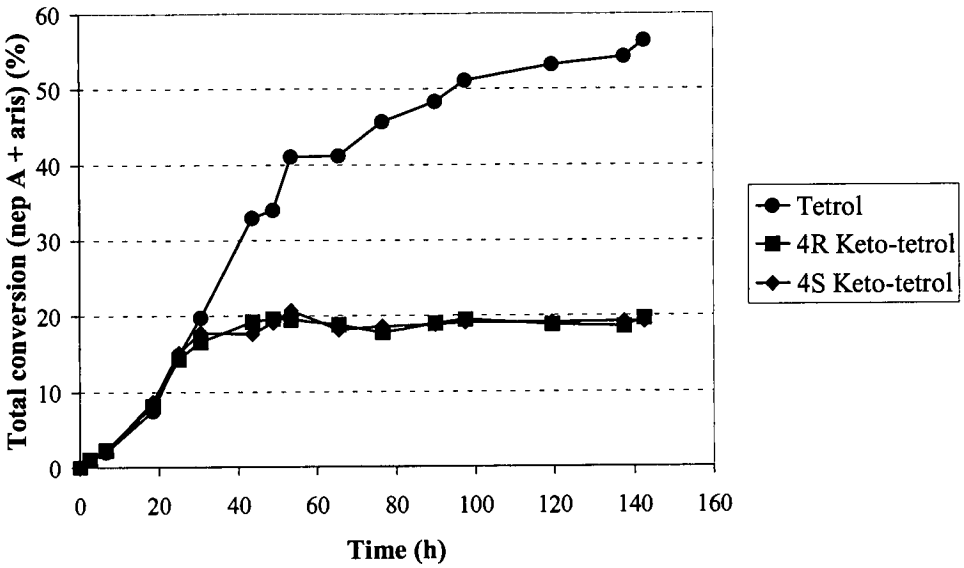
Graphs 2.4a, b and c show that both the 4*R* keto-tetrol **95** and the 4*S* keto-tetrol **116** are converted by *Streptomyces citricolor* to neplanocin A and aristeromycin. Contrary to the previous experiment, the conversion of the 4*S* keto-tetrol **116** to neplanocin A and aristeromycin follows almost exactly the same pattern as for the conversion of the 4*R* keto-tetrol **95**. This result suggests that the halt in conversion, which was observed in the previous feeding experiment, was a result of the small volume of fermentation broth remaining towards the end of the experiment.



Graph 2.4a Conversion to neplanocin A



Graph 2.4b Conversion to aristeromycin



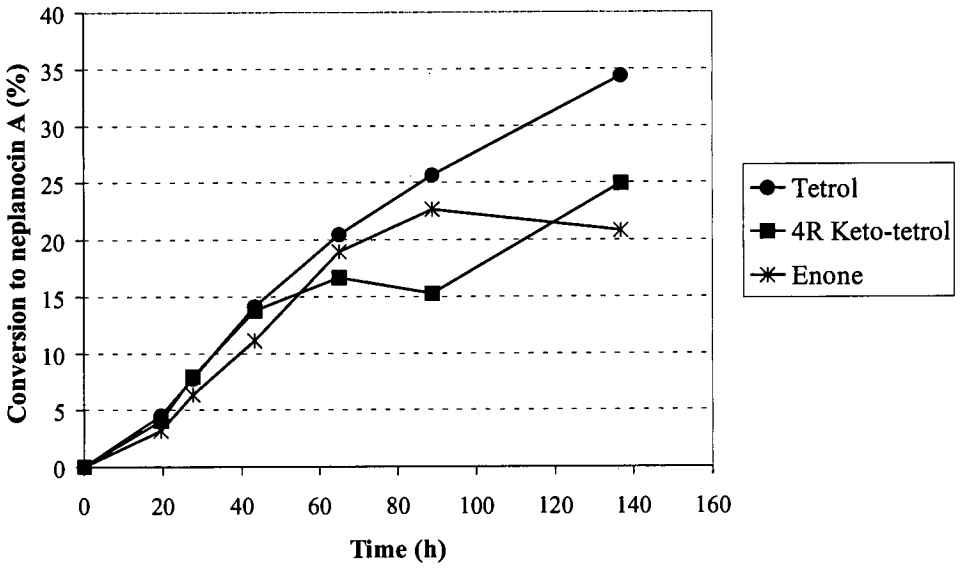
Graph 2.4c Conversion to neplanocin A + aristeromycin

From Graph 2.4a it can be seen that the conversion of the 4*R* and 4*S* keto-tetrol to neplanocin A reaches its maximum (approximately 15%) after incubation for approximately 35 hours. After this time conversion of the keto-tetrol to neplanocin A stops and the amount of neplanocin A decreases as the produced neplanocin A is converted to aristeromycin. Initially the ratio of neplanocin A:aristeromycin is approximately 5:1, however upon prolonged incubation the amount of neplanocin A gradually falls with a concomitant increase in the level of aristeromycin to give a final ratio of approximately 1:1 after incubation for 142 hours.

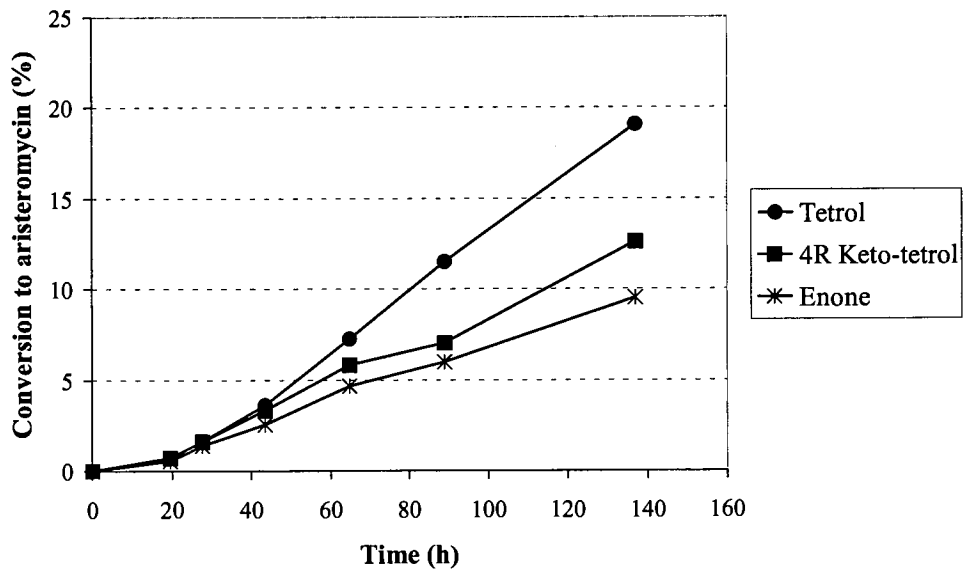
Graph 2.4a shows that the conversion of the tetrol **83a** to neplanocin A reaches its maximum (approximately 30%) after incubation for approximately 53 hours. This level of conversion is much greater that that observed for the keto-tetrol compounds. It was thought that the maximum conversion of the keto-tetrols was less than that for the tetrol **83a** as the keto-tetrols lie further back on the biosynthetic pathway and when they undergo conversion more side reactions occur which results in a lower amount of conversion to neplanocin A and aristeromycin. To test this hypothesis the final experiment involved feeding the synthesised enone **82a** to the *Streptomyces citricolor* mutant.

2.4.5 Keto-tetrol and enone feeding experiment

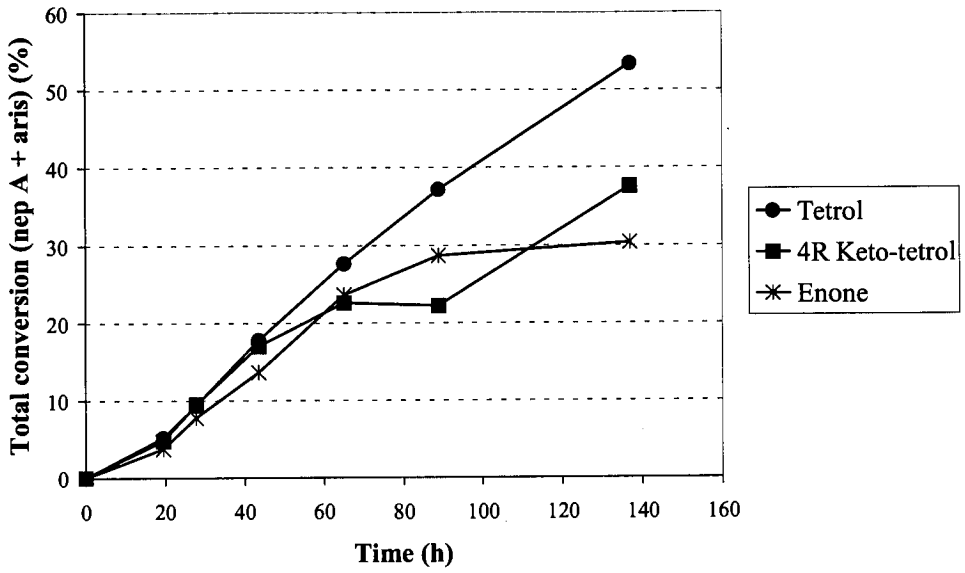
The freshly prepared 4*R* keto-tetrol **95** and enone **82a** were fed to the *Streptomyces citricolor* mutant as well as the tetrol **83a**. The results of these experiments are shown in Graphs 2.5a, b and c.



Graph 2.5a Conversion to neplanocin A



Graph 2.5b Conversion to aristeromycin



Graph 2.5c Conversion to neplanocin A + aristeromycin

In this experiment the 4*R* keto-tetrol **95** was incubated for 96 hours after which time conversion of the keto-tetrol to neplanocin A has ceased. At this point in order to establish whether the organism was still capable of converting the substrate to neplanocin A, a further portion of 4*R* keto-tetrol **95** was added to the fermentation broth. From Graph 2.5a further conversion to neplanocin A was seen after addition of the substrate, which confirms that the organism was still able to convert the substrate to neplanocin A and had simply used up all the initial substrate added and required more to continue conversion to neplanocin A and aristeromycin.

Graph 2.5a shows that conversion of the 4*R* keto-tetrol **95** to neplanocin A reaches its maximum (approximately 17%) after incubation for 65 hours. For the enone **82a** the conversion to neplanocin A reaches its peak (approximately 23%) after incubation for 88 hours. For the tetrol **83a** after incubation for 140 hours the maximum conversion has not yet been obtained. This result confirms the previous hypothesis that since the keto-tetrol is situated further back in the biosynthetic pathway than the enone **82a** and the tetrol **83a** then its maximum conversion is less than for the enone **82a**. Similarly, the maximum conversion of the enone **82a** is less than that observed for the tetrol **83a**.

2.4.6 Conclusion

The results of these feeding studies show that the 4*R* keto-tetrol **95** and its diastereomer 4*S* keto-tetrol **116** are converted by the organism *Streptomyces citricolor* to neplanocin A **5** and aristeromycin **4**. This strongly suggests that both compounds are intermediates on the biosynthetic pathway. However, further studies are required to determine conclusively that these compounds do indeed lie on the biosynthetic pathway.

2.5 Summary of Chapter 2

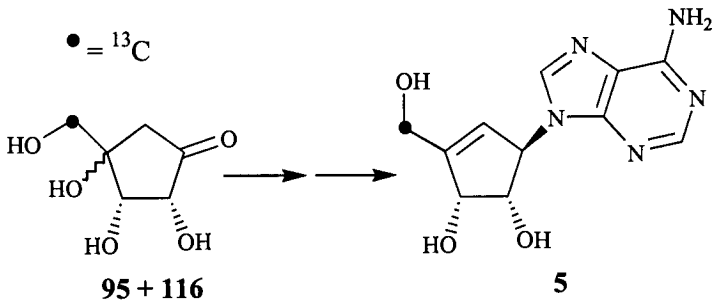
The syntheses of the keto-tetrol **95**, its enantiomer keto-tetrol ***ent*-95** and its diastereomer keto-tetrol **116** have been achieved *via* multi-step syntheses from L-ribose, D-ribonolactone and cyclopentadiene, respectively. Good yields were obtained throughout the synthetic routes except for the reactions that involved the addition of the lithiated [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108**, which resulted in a low overall yield of the desired keto-tetrol being obtained. However, synthesis on a large scale allowed sufficient material to be prepared for use in feeding studies.

The synthesised keto-tetrol compounds were then fed to the *Streptomyces citricolor* mutant CC940. From these studies it was established that the 4*R* keto-tetrol **95** and the 4*S* keto-tetrol **116** were converted by *Streptomyces citricolor* to neplanocin A **5** and aristeromycin **4**. However in order to establish conclusively that these compounds lie on the biosynthetic pathway further studies will have to be performed.

2.6 Future Work

In order to determine whether the 4*R* keto-tetrol **95** and its diastereomer 4*S* keto-tetrol **116** lie on the biosynthetic pathway it will be necessary to prepare the 5-^{13}C labelled keto-tetrols **95** and **116**. These compounds could be prepared from the ^{13}C labelled [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane in which the ^{13}C label

would be introduced using ^{13}C labelled paraformaldehyde. These compounds would then be fed to the *Streptomyces citricolor* CC940 mutant and if the neplanocin A and aristeromycin produced contains the ^{13}C label at the 5' position then the keto-tetrol compounds would be deemed to lie on the biosynthetic pathway (Scheme 2.16).



95 + 116 **5**

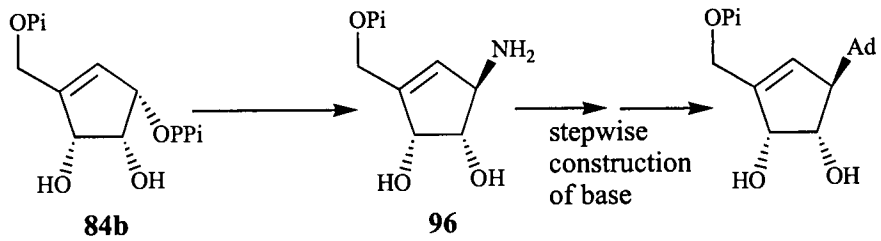
Scheme 2.16

The experiment outlined in Scheme 2.16 would not rule out the possibility of chemical elimination (of the keto-tetrols **95** and **116** to the enone **82a**) taking place. Therefore it would be important to develop an assay in which the keto-tetrol, enone and tetrol could be detected as well as neplanocin A and aristeromycin. This assay would provide more information as to what is happening during the feeding experiments when the keto-tetrol is converted to neplanocin A and aristeromycin. Development of an HPLC assay to do this was investigated however due to time constraints no suitable assay was found.

3 Investigations into the Incorporation of Adenine into Neplanocin A – Identification of Phosphorylated Intermediates

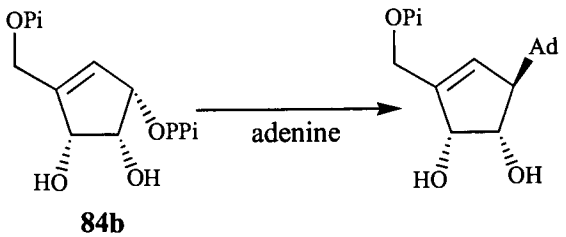
Two possible methods for the introduction of the adenine base into neplanocin A were considered:

i. *de novo* purine biosynthesis: in which the adenine ring is biosynthesised by stepwise construction of the appropriate precursors onto a carbocyclic analogue of 5-phosphoribosyl pyrophosphate containing a pre-existing amino group **96** as shown in Scheme 3.1.



Scheme 3.1

ii. purine salvage pathway: in which the adenine ring is incorporated intact onto a carbocyclic analogue of 5-phosphoribosyl pyrophosphate **84b** as outlined in Scheme 3.2.

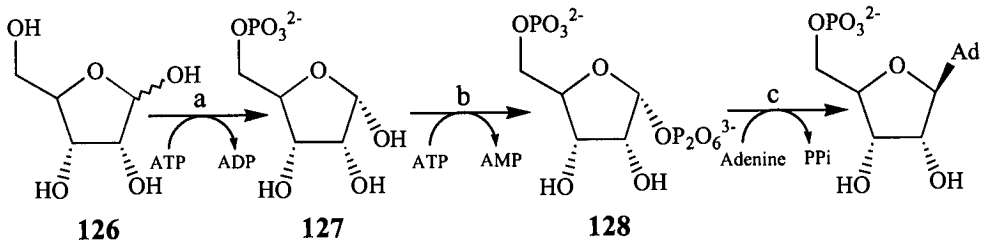


Scheme 3.2

Previous studies which are discussed in Section 1.3.1 (c) strongly suggest that the operation of a *de novo* biosynthesis is unlikely and that direct incorporation of adenine is the major route to aristeromycin **4** and neplanocin A **5**.

By analogy with the salvage pathway for the biosynthesis of natural purine nucleosides in which D-ribose **126** is converted to D-ribose-5-phosphate **127** and then to 5-phosphoribosyl pyrophosphate **128** in which adenine is incorporated intact, this leads to the proposal that adenine is incorporated into neplanocin A *via* the pathway shown in Figure 3.1, in which the pyrophosphate **84b** is proposed to be the immediate precursor of neplanocin A. The tetrol **83a** is a known intermediate on the biosynthetic pathway.

Salvage biosynthesis of purine nucleosides



a. ribokinase; b. phosphoribosyl pyrophosphate synthetase;

c. adenine phosphoribosyl transferase

Proposed mechanism for the introduction of adenine into neplanocin A

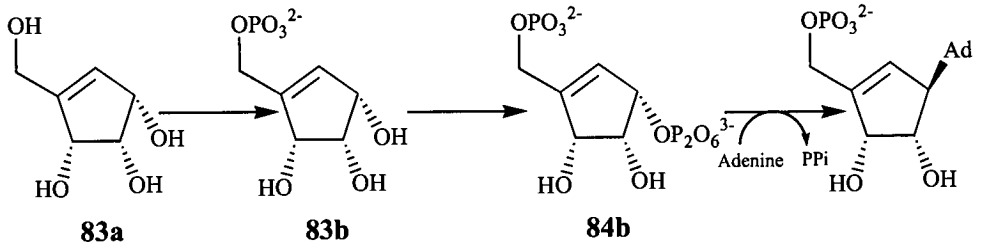
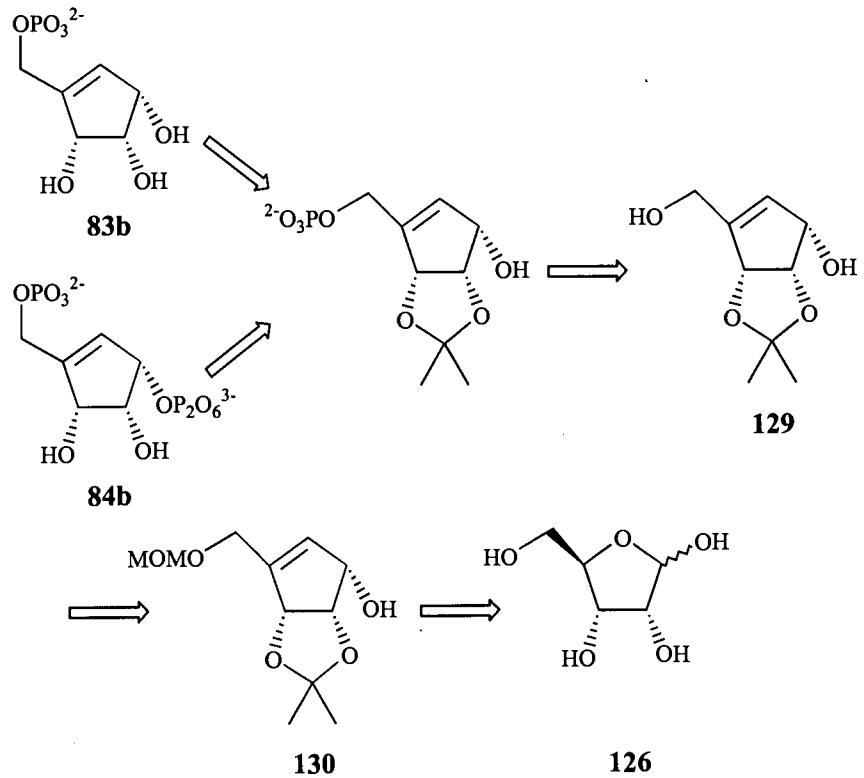


Figure 3.1

The aim of this work was therefore to prepare the proposed phosphorylated intermediates, the 5-phosphate **83b** and the pyrophosphate **84b**, and establish whether they lie on the biosynthetic pathway. These compounds would be challenging synthetic targets due to the known instability of allylic phosphates and pyrophosphates.

3.1 Retrosynthetic Analysis

It was envisioned that the proposed phosphorylated and pyrophosphorylated intermediates could be prepared according to the retrosynthetic plan shown in Scheme 3.3, in which the key intermediate is the methoxymethyl-protected alcohol **130**, the synthesis of which from D-ribose **126** has been reported in the literature.[97]



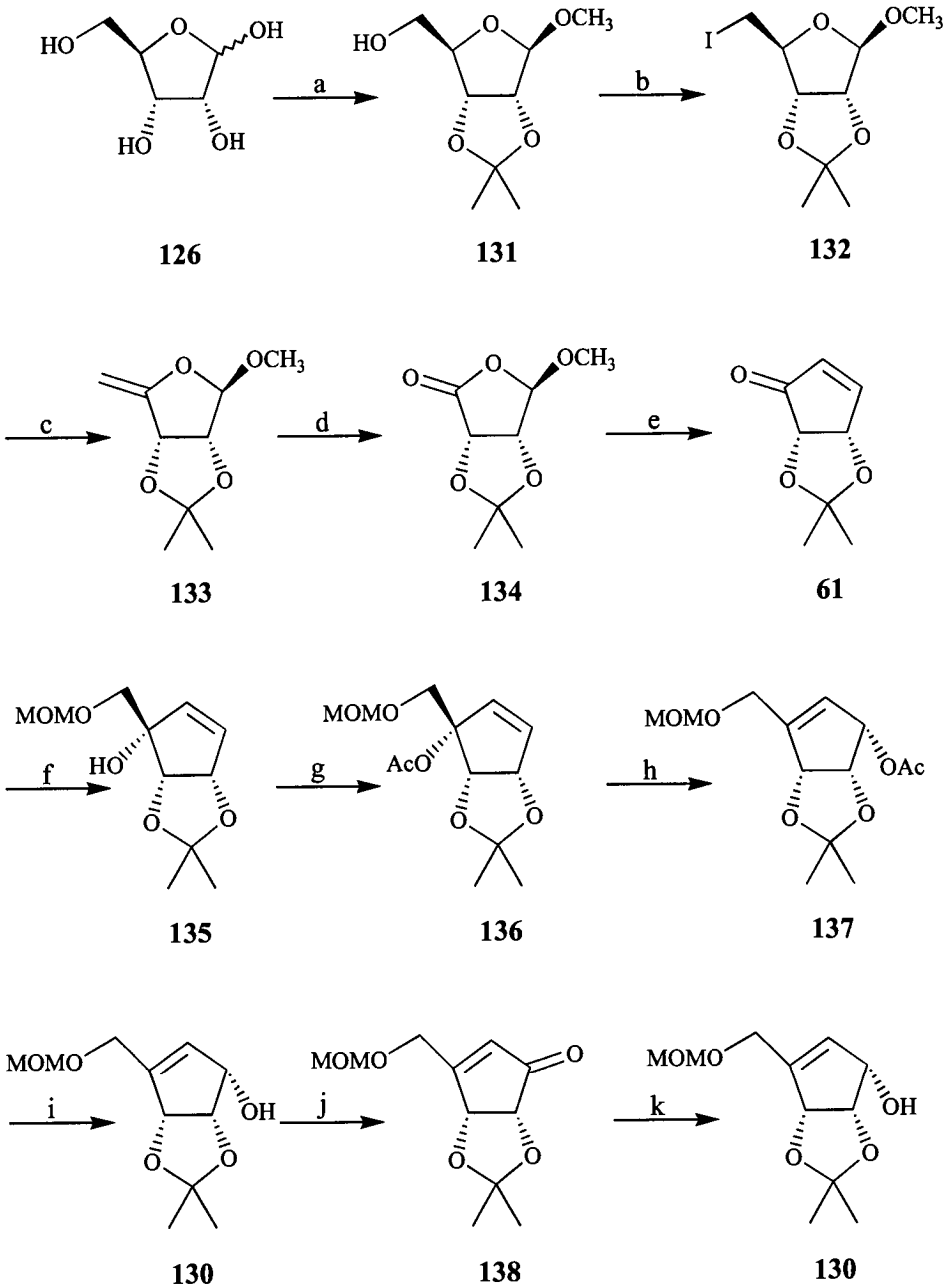
Scheme 3.3

The initial goal was to repeat the synthesis of the methoxymethyl-protected alcohol **130** from D-ribose **126** and to use this compound to prepare the tetrol **83a**, which is a known intermediate on the biosynthetic pathway and is required as a control compound for use in feeding studies.

3.2 Synthesis of Tetrol 83a

3.2.1 Synthesis *via* methoxymethyl-protected compounds

The methoxymethyl-protected alcohol (1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(methoxymethoxy)methyl]-cyclopent-4-en-1-ol **130**, was prepared from D-ribose **126** as shown in Scheme 3.4.

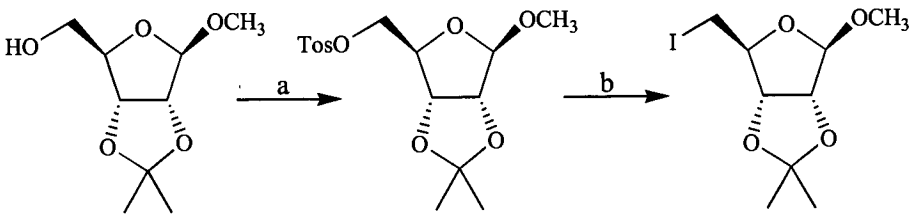


Scheme 3.4 *Reagents and conditions:* a. DMP, acetone, $HClO_4$, methanol, 81%; b. I_2, imidazole, PPh_3; c. DBU, THF, reflux, 64% 2 steps; d. OsO_4, $NaIO_4$, aq. THF, 67%; e. $(MeO)_2P(O)Me$, *n*-BuLi, THF, -78°C, 49%; f. Bu_3SnCH_2OMOM **110**, *n*-BuLi, THF, -78°C, 72%; g. Ac_2O, pyridine, DMAP, CH_2Cl_2, 89%; h. $PdCl_2(MeCN)_2$, benzoquinone, THF, reflux, 66%; i. K_2CO_3, MeOH, 87%; j. IBX, DMSO, 87%; k. $NaBH_4$, $CeCl_3.7\ H_2O$, MeOH, 86%.

D-Ribose **126** was protected as its methyl glycoside and 2,3-acetonide derivative **131** in 81% yield, using DMP, perchloric acid and methanol in acetone.[63]

The protected ribose **131** was then converted to the iodide **132** using iodine, imidazole and triphenylphosphine. The iodide **132** obtained was thought to be unstable therefore the crude iodide was immediately eliminated to the enol ether **133** in 64% yield by refluxing in DBU. Problems were encountered in the work-up of this "one-pot" reaction due to the presence of the triphenylphosphine oxide by-product, which made the residue from which the iodide was to be extracted very thick. This resulted in the yield of enol ether **133** being less than expected.

The preparation of the iodide **132** *via* the tosylate **139** as shown in Scheme 3.5 was investigated. However the overall yield of enol ether **133** obtained was only 45% and due to the large amount of sodium iodide required the reaction was not applicable to a large scale.



131 **139** **132**

Scheme 3.5 *Reagents and conditions:* a. TsCl, pyridine, DMAP, CH_2Cl_2; b. NaI, acetone, reflux.

Sodium periodate-osmium tetroxide oxidation of the enol ether **133** gave the lactone **134** in 67% yield.

The subsequent cyclisation of the lactone **134** to the enantiomerically pure cyclopentenone **61** caused problems in this synthetic pathway. The reaction was extremely unreliable and gave low and variable yields of 0-35%. When this reaction was carried out following the procedure described by Borchardt *et al.*[98] the yields of cyclopentenone **61** obtained were 8% and 13%. It was found however, that quenching the reaction at low temperature increased the yield to 49%. Moreover, the reaction was less capricious and more reliable.

The proposed mechanism for this cyclisation reaction *via* a Wadsworth-Emmons type mechanism is shown in Figure 3.2.

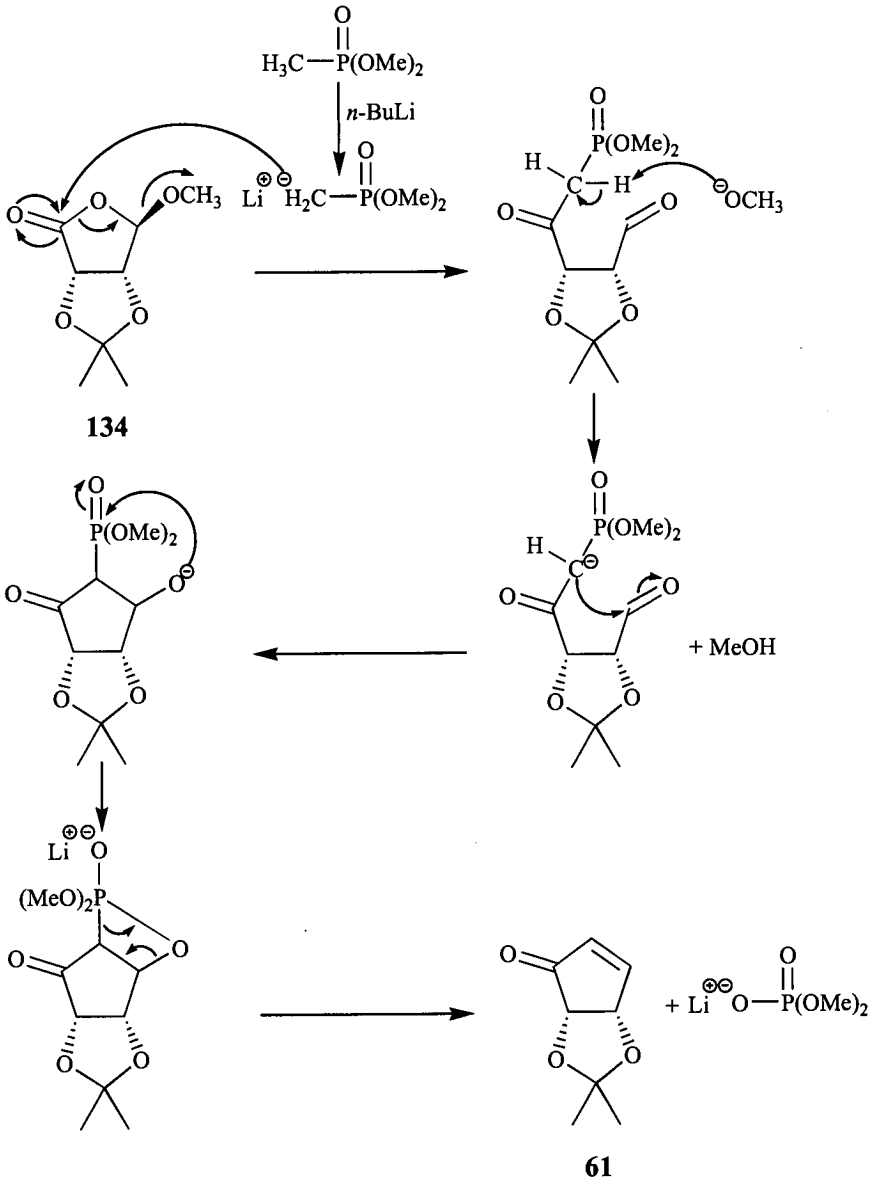
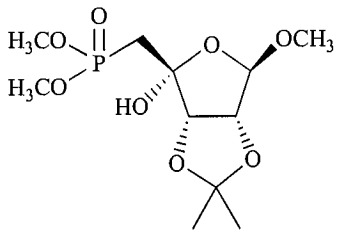


Figure 3.2 Proposed mechanism for the cyclisation reaction

Deprotonation of dimethyl methylphosphonate using one equivalent of *n*-butyllithium yields the corresponding anion, which adds to the carbonyl group of the lactone **134** resulting in the opening of the lactone ring and elimination of methoxide to give an acyclic intermediate. This intermediate then undergoes base-promoted cyclisation to the cyclopentenone **61** by the methoxide generated *in situ*.

In an attempt to improve the yield and reliability of this procedure, the reaction mixture was quenched at -78°C by the addition of saturated aqueous ammonium chloride solution. This resulted in no desired product being formed; instead a 61%

yield of the novel phosphonate **140**, which is proposed to be the first formed intermediate in the reaction, was obtained. The structure and stereochemistry of the phosphonate **140** was confirmed by an X-ray crystal structure.‡



140

It was hoped that treatment of the phosphonate **140** with base would result in formation of the desired cyclopentenone **61**. However, treatment of the phosphonate **140** with sodium methoxide, sodium hydride, *n*-butyllithium and sodium bis(trimethylsilyl)amide under a variety of conditions resulted in decomposition of the starting phosphonate **140** and no desired cyclopentenone **61** being obtained.

Enantiomerically pure cyclopentenones of this type are important synthetic intermediates. For example, they have been used in prostaglandin synthesis and in the preparation of carbocyclic nucleosides, the most recent examples of which are discussed in Section 1.2.2 (b) (i). This has resulted in many attempts to synthesise these compounds, which have included their preparation from cyclopentadiene,[93] D-ribonolactone,[89,98] D-ribose and D-lyxose,[63] D-mannose,[98] fulvene,[99] toluene and chloro- and bromo-benzene.[100] However all these syntheses have their disadvantages.

Recently, a reliable and efficient synthetic route to the cyclopentenone **61** from cyclopentadiene **36** has been developed within the Turner group, using a revised procedure to that described by Johnson and Penning.[93] This pathway which is described in detail in Section 2.2.2 provides a convenient, alternative route to the enantiomerically pure cyclopentenone **61**.

‡ This crystal structure was determined by an Honours project student working under the supervision of Dr Lindsay Sawyer in the Institute of Cell and Molecular Biology, the University of Edinburgh.

Following work described by Johnson and Medich,[101] the enone **61** was converted to the protected diol **135** using [(methoxymethoxy)methyl]tri-*n*-butylstannane **110**. The stannane **110** was prepared using a procedure reported by McGarvey *et al.*[102] in which (tri-*n*-butylstannyl)methanol was alkylated with chloromethyl methyl ether as shown in Scheme 3.6.

$$Bu_3SnH \xrightarrow{a} Bu_3SnLi \xrightarrow{b} Bu_3SnCH_2OH \xrightarrow{c} Bu_3SnCH_2OCH_2OCH_3$$

110

Scheme 3.6 *Reagents and conditions:* a. diisopropylamine, *n*-BuLi, THF, 0°C; b. para-formaldehyde; c. dimethylaniline, CH_2Cl_2, chloromethyl methyl ether , 0°C, 85% 3 steps.

Treatment of Bu_3SnCH_2OMOM **110** with *n*-butyllithium gave the hydroxymethyl anion equivalent which adds to the carbonyl group of the cyclopentenone **61** to give the protected diol **135** in 72% yield.

Acetylation of the allylic hydroxyl group of the protected diol **135** using acetic anhydride, pyridine and a catalytic amount of DMAP gave the ester **136** in 89% yield.

Allylic acetates such as ester **136** are known to rearrange upon treatment with catalytic $PdCl_2(CH_3CN)_2$ in THF to give the corresponding acetates with complete retention of stereochemistry.[103] The palladium catalysed [3,3]-sigmatropic rearrangement of ester **136** to acetate **137** caused problems in this synthetic sequence. Examination of both the ^1H and ^{13}C NMR spectra of the acetate **137** showed the presence of a minor contaminant which could not be removed by silica column chromatography. Various catalysts and conditions were used in the reaction with a view to eliminating the impurity and optimising the reaction, including the use of Pd(II) acetate and tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct. From the results of these investigations it was observed that the only catalyst which gave the desired product was the bis(acetonitrile)dichloropalladium(II) catalyst and that the greatest yield for the reaction was obtained when refluxing in THF for 6 hours. Examination of the ^1H and ^{13}C NMR spectra of the acetate **137** product obtained in this case showed significantly less impurity than when the reaction mixture was refluxed for 8 hours or more. It was not possible to identify the impurity that was present, however one possibility is a chlorinated adduct derived

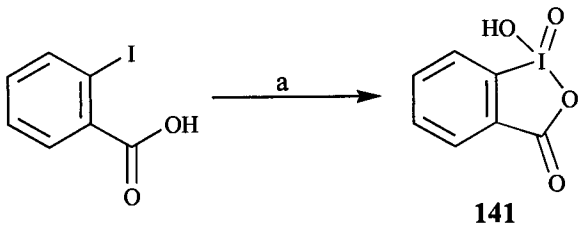
from incorporation of chlorine into the compound from the bis(acetonitrile)dichloro-palladium(II) catalyst used.

The impure acetate **137** was deacetylated using anhydrous potassium carbonate in methanol to give the alcohol **130**, which by examination of the ^{1}H NMR spectrum showed the presence of the same impurity that was present in the acetate starting material **137**.

In order to eliminate the impurity and obtain the alcohol **130** in a pure form it was necessary to introduce the oxidation-reduction sequence shown in Scheme 3.4.[97]

In this sequence, as an alternative to the reported PCC oxidation,[97] the impure alcohol **130** was oxidised using IBX **141** in DMSO to give the enone **138** in 87% yield. The enone **138** from this reaction could be obtained in pure form after purification by column chromatography.

IBX **141** was prepared from the inexpensive, commercially available reagents as shown in Scheme 3.7.[104]

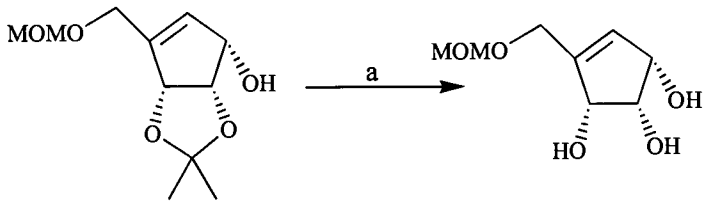


141

Scheme 3.7 *Reagents and conditions:* a. $KBrO_3$, H_2SO_4, 55°C to 84°C, 51%.

Following the procedure reported by Bestmann and Roth,[105] the carbonyl group of the enone **138** was selectively reduced using sodium borohydride and cerium(III) chloride heptahydrate to give the desired MOM-protected alcohol **130** in 86% yield.

In order to prepare the proposed phosphorylated intermediates it was necessary to selectively deprotect the MOM group in the presence of the acetonide group. However, treatment of the MOM-protected alcohol **130** with Amberlite IR 120 (H) in methanol and water showed that the acetonide-protecting group was preferentially cleaved to give the MOM-protected triol **142** as shown in Scheme 3.8.



130 **142**

Scheme 3.8 *Reagents and conditions:* a. Amberlite IR 120 (H), MeOH, H_2O, 57%.

Attempts to selectively deprotect the MOM group of the MOM-protected alcohol **130** were carried out using the following conditions:

i. Trifluoroacetic acid:dichloromethane (1:1), room temperature.[106]
   Deprotection of both the acetonide group and the MOM group, the acetonide group being cleaved first.

ii. *p*-Toluenesulfonic acid, methanol, room temperature.
   The acetonide group was deprotected first followed by cleavage of the MOM group. However, deprotection was much slower than observed for the TFA deprotection. After stirring at room temperature for two days starting material still remained.

iii. Pyridinium *p*-toluenesulfonate, propan-2-ol, room temperature.
   Stirring at room temperature for fourteen days resulted in no reaction taking place.

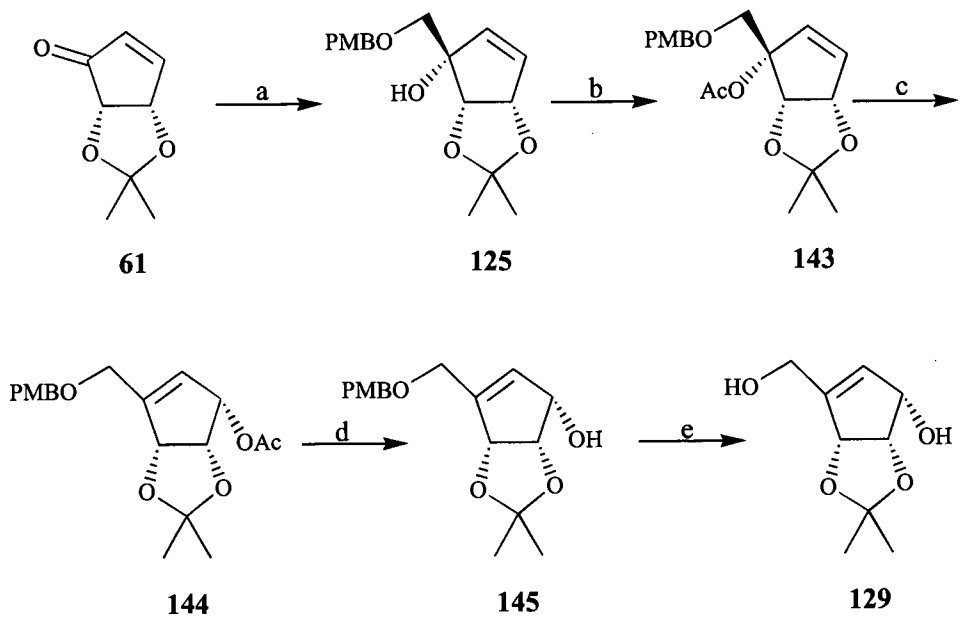
From these results, and the lack of precedence in the literature, it appeared that the selective deprotection of the MOM group in the presence of the acetonide moiety would not be possible. Therefore the preparation of the desired phosphorylated intermediates *via* this synthetic pathway was therefore abandoned.

The MOM-protected alcohol **130** could however be used to prepare the tetrol **83a** in 52% yield by stirring in Amberlite IR 120 (H) for 4 days.

3.2.2 Synthesis *via para*-methoxybenzyl-protected compounds

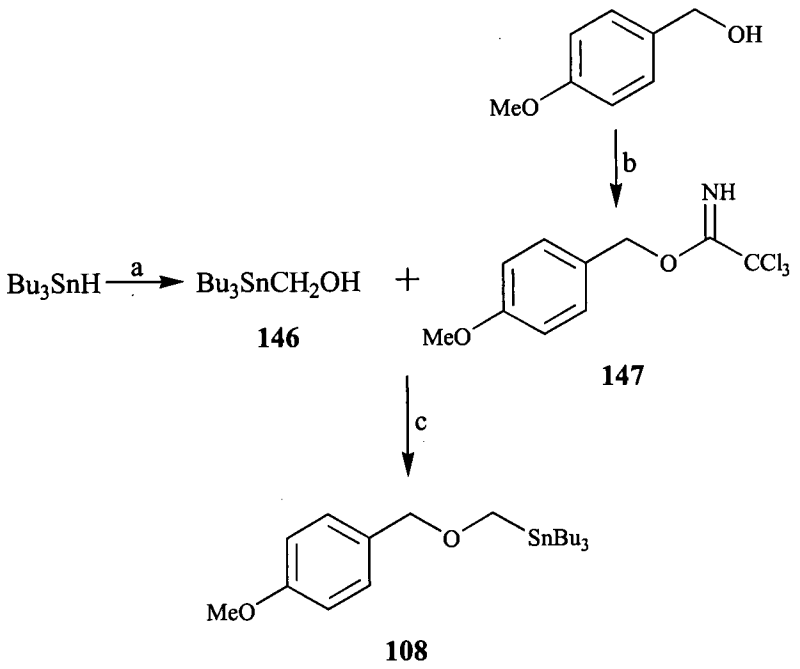
As the MOM protecting group could not be selectively deprotected it had to be replaced with a more suitable protecting group. When replacing the MOM group it was necessary to consider that not only did the new protecting group need to be orthogonal to the acetonide group but it must also be able to be introduced *via* the corresponding tri-*n*-butylstannane.

At this time Parry *et al.*[107] published a paper that described their investigations into the conversion of carbocyclic analogues of D-ribose-5-phosphate to the corresponding carbocyclic PRPP analogues using 5-phosphoribosyl α-1-pyrophosphate synthetases. In this paper the synthesis of the 5-phosphate **83b** from the cyclopentenone **61** was described using a procedure similar to that proposed by us but with the replacement of the methoxymethyl group with the *para*-methoxybenzyl group. It was therefore decided to replace the MOM group with the PMB group and the synthesis was carried out as shown in Scheme 3.9.



Scheme 3.9 *Reagents and conditions:* a. Bu_3SnCH_2OPMB **108**, *n*-BuLi, THF, -78°C, 73%; b. Ac_2O, pyridine, DMAP, CH_2Cl_2, 96%; c. $PdCl_2(MeCN)_2$, benzoquinone, THF, 63%; d. K_2CO_3, MeOH, 61%; e. DDQ, CH_2Cl_2:H_2O (20:1), 38%.

[(*p*-Methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** was prepared as shown in Scheme 3.10.



Scheme 3.10 *Reagents and conditions:* a. i. LDA, ii. $(CH_2O)_n$; b. i. NaH, ii. Cl_3CCN; c. CF_3SO_3H, 42% overall.

(Tri-*n*-butylstannyl)methanol **146** was prepared according to the procedure reported by Still[108] in which tri-*n*-butyltin hydride is deprotonated with LDA and then alkylated by the addition of paraformaldehyde. Meanwhile the commercially available *p*-methoxybenzyl alcohol was converted to the (*p*-methoxybenzyl)trichloroacetimidate **147** by treatment with sodium hydride and trichloroacetonitrile. These two fragments were then coupled together in the presence of triflic acid to give the desired [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** after purification by column chromatography and then distillation in 42% overall yield. This provided a more convenient route to the [(*p*-methoxybenzyloxy)methyl]-tri-*n*-butylstannane **108** than that described by Parry[107] (by avoiding the use of sulfur containing compounds). The attempted preparation of the [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** using a procedure analogous to that used for the preparation of [(methoxymethoxy)methyl]tri-*n*-butylstannane **110** in which *p*-methoxybenzyl chloride was added to (tri-*n*-butylstannyl)methanol **146** was unsuccessful and produced no desired product.

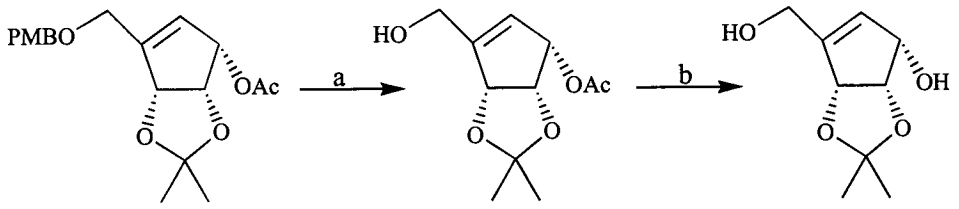
Lithiation of the [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** gave the hydroxymethyl anion equivalent which added in a 1,2-fashion to the cyclopentenone **61** to give the PMB-protected alcohol **125** in 73% yield.

The protected alcohol **125** was then acetylated using acetic anhydride, pyridine and a catalytic amount of DMAP to give the ester **143** in 96% yield.

As for the MOM-protected ester **136**, the PMB-protected ester **143** underwent the palladium catalysed allylic rearrangement to give the acetate **144** in 63% yield. Examination of the ^{1}H and ^{13}C NMR spectra of the acetate **144** showed the presence of minor traces of the corresponding impurity that was present in the MOM-protected acetate **137**.

The impure acetate **144** was deacetylated by treatment with anhydrous potassium carbonate in methanol to give the protected alcohol **145** in 61% yield. Unlike the MOM-protected alcohol **130**, the PMB-protected alcohol **145** was obtained in pure form and contained none of the impurity that was present in the acetate **144** starting material. Therefore the oxidation-reduction sequence carried out for the MOM-protected compounds did not need to be performed.

The PMB group was then successfully selectively deprotected by treatment with DDQ to give the acetonide protected diol **129** in 38% yield. It was thought that the poor yield was a result of the desired product being very water-soluble and as a result extraction of the protected diol **129** from the aqueous layer during work-up was very difficult. Therefore it was necessary to alter the deprotection steps as shown in Scheme 3.11.



144 **148** **129**

Scheme 3.11 *Reagents and conditions:* a. DDQ, CH_2Cl_2:H_2O (20:1), 84%; b. NH_3 sat. MeOH, 96%.

Selective deprotection of the PMB group from the impure acetate **144** in the presence of the acetonide and acetate groups was achieved in 84% yield by treatment

with DDQ. The alcohol **148** obtained was pure and contained none of the impurity that was present in the acetate **144** starting material.

The acetate group was then removed from the alcohol **148** by stirring in ammonia saturated methanol to give the acetonide protected diol **129** in 96% yield. The use of this reagent, which can simply be removed by evaporation under vacuum, meant that there was no problem with the desired product being lost during aqueous work-up.

The tetrol **83a**, which has been used in the feeding studies as discussed in Section 2.4, was subsequently obtained in 94% yield by deprotection of the acetonide group of **129** by treatment with trifluoroacetic acid.

Having established a synthetic route to the cyclopentenol type compounds, the synthesis of the proposed phosphorylated intermediates was then investigated.

3.3 Synthesis of Phosphorylated Intermediates

Monoalkyl phosphates are recognised to play important roles in many biological processes and as a result several chemical procedures have been developed for the conversion of alcohols into their corresponding phosphate esters.[109] One such procedure that has recently joined the P^V phosphorylation strategies as a popular method for the preparation of phosphate monoesters is the use of P^{III} phosphoramidite reagents in which the selection of the phosphate protecting group is of paramount importance.[110]

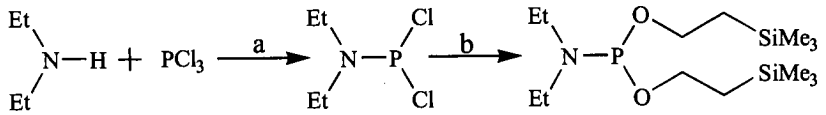
In the synthesis of the desired phosphorylated intermediates the choice of phosphate protecting group is governed by the requirements that the protecting group must:

i. be able to be deprotected under mild conditions without causing reduction of the double bond of the cyclopentene ring.
ii. be stable to the other reagents and conditions used in subsequent reactions in the synthetic pathway.

In the synthetic route to the 5-phosphate **83b** recently reported by Parry,[107] the 2-(trimethylsilyl)ethyl protecting group was found to satisfy these conditions, its introduction being *via* the bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150**. The 2-(trimethylsilyl)ethyl group can be cleaved by fluoride anion *via* a β-elimination mechanism to give ethene and the corresponding trimethylsilyl fluoride.[111] Therefore, the use of this reagent and the 2-(trimethylsilyl)ethyl protecting group was employed in the synthesis of the desired phosphorylated intermediates.

3.3.1 Preparation of phosphorylating agent 150

Bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150** was prepared using the procedure reported by Parry[107] which is outlined in Scheme 3.12.



149 **150**

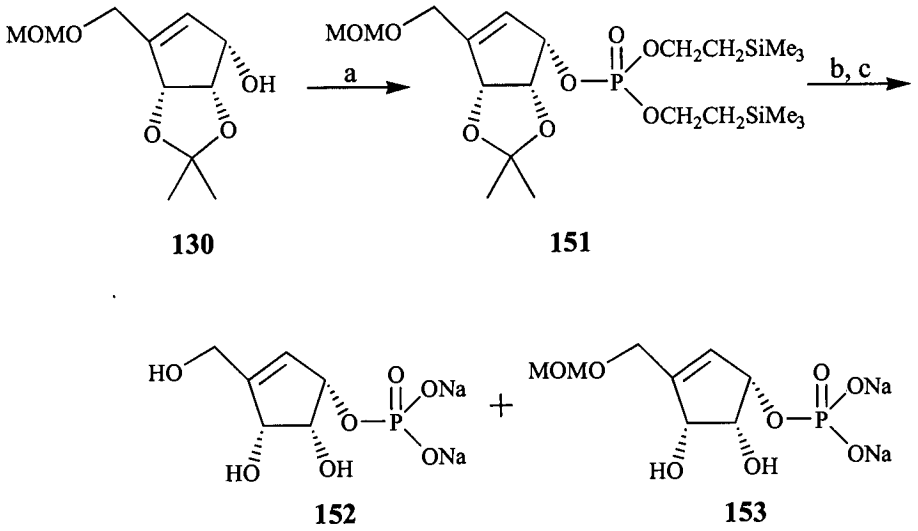
Scheme 3.12 *Reagents and conditions:* a. THF, <0°C to room temperature, 53%; b. 2-(trimethylsilyl)ethanol, triethylamine, THF, 0°C to room temperature, 41%.

N,*N*-Diethylphosphorochloridite **149** was prepared according to the procedure published by Perich and Johns[109] by the reaction of diethylamine with phosphorous trichloride. Treatment of the *N*,*N*-diethylphosphorochloridite **149** with 2 equivalents of 2-(trimethylsilyl)ethanol in the presence of triethylamine gave the desired bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150** in 41% yield. This reagent which has also been prepared in an alternative synthesis reported by Freeman *et al.*[110] is stable for over 12 months when stored at <0°C.

3.3.2 Synthesis of 1-phosphate 152

(a) Synthesis *via* methoxymethyl-protected compounds

In order to establish the viability of the synthetic procedures and experimental techniques used, the MOM-protected alcohol **130** was firstly converted to the 1-phosphate **152** as shown in Scheme 3.13. Although the 1-phosphate **152** is not a proposed intermediate on the biosynthetic pathway it was viewed as a useful substrate in feeding studies.



Scheme 3.13 *Reagents and conditions:* a. i. $(Me_3SiCH_2CH_2O)_2PNEt_2$ **150**, 1*H*-tetrazole, THF; ii. MCPBA, CH_2Cl_2, -40°C to room temperature, 55%; b. 48-51% HF:MeCN:H_2O (10:85:15); c. 1 M NaOH, H_2O.

The secondary hydroxyl group of the MOM-protected alcohol **130** was phosphorylated using bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150** in the presence of 1*H*-tetrazole. Without isolation, the intermediate phosphite ester formed was oxidised by the addition of MCPBA to give the 2-(trimethylsilyl)ethyl protected phosphate triester **151** in 55% yield.

The final step in the synthesis, which involved the deprotection of the protecting groups and subsequent formation of the sodium salt of the phosphate ester, caused problems in the synthesis.

Preparation of the sodium salt of the deprotected phosphate using the literature procedure described by Parry[107] did not result in the sodium salt being formed. Model studies on sodium salt formation were carried out using the commercially available α-D-glucose-1-phosphate, which is purchased as its disodium salt. The disodium salt was firstly converted to the acid form by stirring in AG 50W-X2 resin

for two hours. Confirmation that the acid form had indeed been prepared was obtained by the ^{31}P NMR spectrum (δ_P of disodium salt = 3.20, δ_P of acid form = 0.08). It was then attempted to convert the acid form to the sodium salt using AG 50W-X2 resin, sodium form and Amberlite IR 120, sodium form using both a column method and by stirring in the resin, however none of these methods resulted in the sodium salt being formed.

The preparation of the sodium salt could however be achieved by careful titration of the acid form with sodium hydroxide.

As an alternative to the preparation of a disodium salt the formation of the di(cyclohexylammonium) salt was successfully achieved by stirring the acid form in cyclohexylamine.

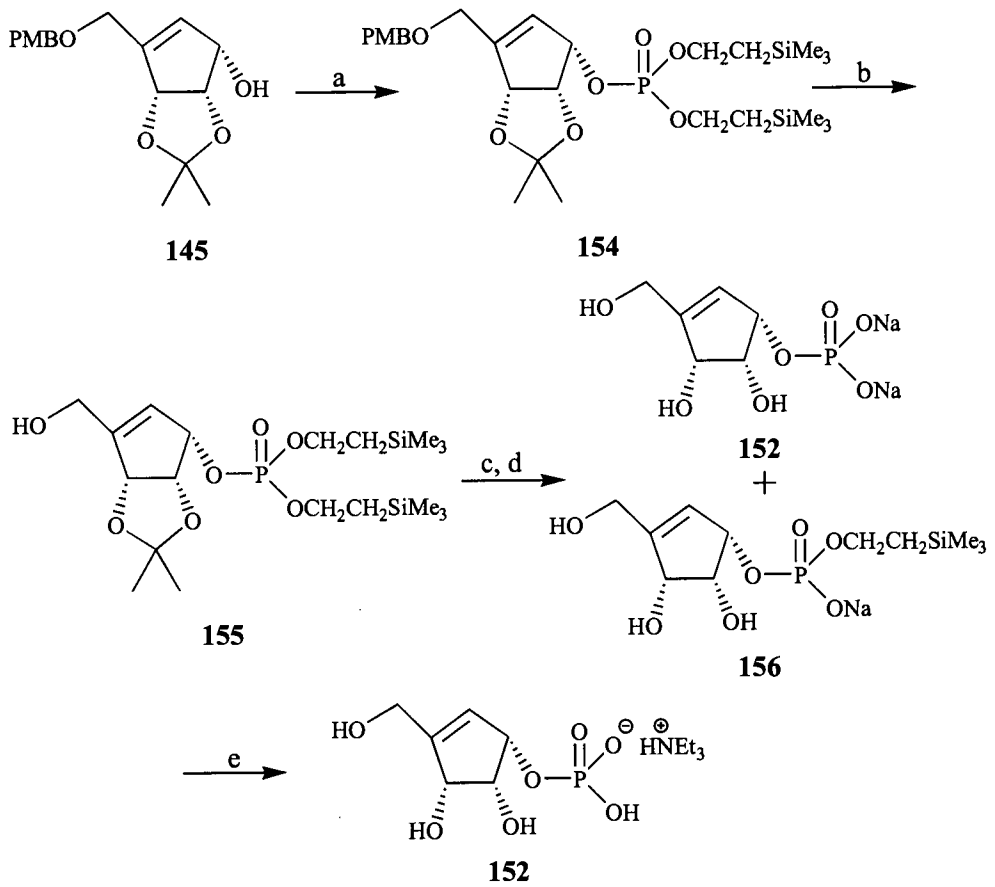
The protected phosphate **151** was then deprotected by treatment with 48-51% HF:MeCN:H_2O (10:85:15) stirring for 24 hours before conversion to the disodium salt by titration with 1 M NaOH. However, ^{1}H, ^{13}C, ^{31}P NMR and mass spectrometry showed that the product of this reaction was a mixture of the desired fully deprotected phosphate (disodium salt) **152** and the MOM-protected phosphate (disodium salt) **153**. Attempts to deprotect the MOM protecting group by stirring in Amberlite IR 120 (H) and trifluoroacetic acid were unsuccessful and resulted in the decomposition of the phosphorylated compound.

(b) Synthesis *via para*-methoxybenzyl-protected compounds

As the MOM group could not be deprotected without decomposition taking place it was decided to prepare the 1-phosphate **152** *via* the PMB-protected compounds.

The desired 1-phosphate **152** was prepared from the PMB-protected alcohol **145** as shown in Scheme 3.14.

As for the MOM-protected alcohol **130**, the secondary hydroxyl group of the PMB-protected alcohol **145** was phosphorylated using bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150** in the presence of 1*H*-tetrazole. The intermediate phosphite ester formed was oxidised using MCPBA to give the protected phosphate **154** in 83% yield.



Scheme 3.14 *Reagents and conditions:* a. i. $(Me_3SiCH_2CH_2O)_2PNEt_2$ **150**, 1*H*-tetrazole, THF; ii. MCPBA, CH_2Cl_2, -70°C to room temperature, 83%; b. DDQ, CH_2Cl_2:H_2O (20:1), 94%; c. 48-51% HF:MeCN:H_2O (10:85:15); d. 0.1 M NaOH, H_2O; e. Ion-exchange chromatography, 60%.

The PMB group of the protected phosphate **154** was then selectively deprotected using DDQ to give the protected phosphate **155** in 94% yield.

Deprotection of the protected phosphate **155** by stirring in a solution of 48-51% HF:MeCN:H_2O (10:85:15) for 7 hours and then conversion to the sodium salt by titration with sodium hydroxide gave a mixture of the desired fully deprotected phosphate **152** and the phosphate with one 2-(trimethylsilyl)ethyl group still attached **156**. This was contrary to the literature procedure described by Parry[107] who successfully deprotected both 2-(trimethylsilyl)ethyl groups by stirring in a solution of 48% HF:MeCN:H_2O (5:85:15) for 5 hours.

Freeman *et al.*[110] reported that both 2-(trimethylsilyl)ethyl protecting groups could be deprotected by stirring the protected phosphate triester in hydrofluoric acid for 24 hours. However stirring the acetonide protected phosphate **155** in 48-51%

HF:MeCN:H_2O for 24 hours resulted in cleavage of the phosphate group taking place. When the MOM-protected phosphate **151** was subjected to these conditions cleavage of the phosphate group did not appear to occur, the reason for this is unknown however it may be a result of the differing solubilities of the compounds.

In the literature it was reported that the use of alternative fluoride anion sources such as tetrabutylammonium fluoride, lithium tetrafluoroborate, ammonium fluoride and trifluoroacetic acid resulted in either the deprotection reaction being unsuccessful or only one 2-(trimethylsilyl)ethyl protecting group being removed.[110] Therefore the use of these methods for the deprotection of the protected phosphate **155** was not attempted.

The hydrofluoric acid deprotection reaction was carried out under a variety of conditions, the optimum of which was determined to be stirring in a solution of 48-51% HF:MeCN:H_2O (5-10:85:15) for 6 to 7 hours which gave a mixture of the fully deprotected phosphate **152** and the phosphate with one 2-(trimethylsilyl)ethyl group **156** still attached. These compounds could however be easily separated by ion-exchange chromatography using triethylammonium bicarbonate buffer to give the desired 1-phosphate **152** as its triethylammonium salt in 60% yield.

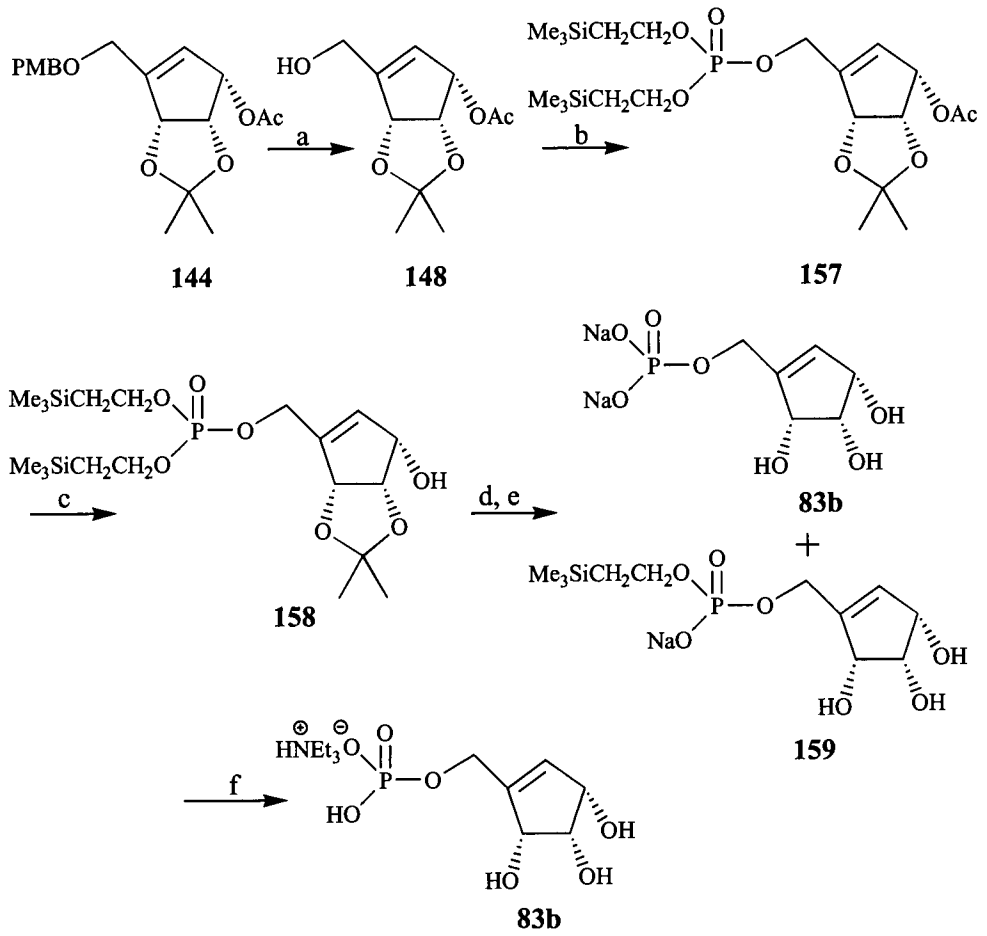
3.3.3 Synthesis of 5-phosphate 83b

Having established a synthetic route to the 1-phosphate **152**, the 5-phosphate **83b** was prepared from the PMB-protected acetate **144** using a similar synthetic pathway, as shown in Scheme 3.15.

The PMB protecting group of the PMB-protected acetate **144** was selectively deprotected in the presence of the acetate and acetonide protecting groups by treatment with DDQ.

The primary hydroxyl group of the resulting acetonide protected acetate **148** was then phosphorylated using bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150** in the presence of 1*H*-tetrazole. As for the previous syntheses the intermediate phosphite ester was oxidised using MCPBA to give the protected phosphate **157** in 85% yield.

Exposure of the protected phosphate **157** to ammonia saturated methanol resulted in deprotection of the acetate group and formation of the protected alcohol **158** in 73% yield.



Scheme 3.15 *Reagents and conditions:* a. DDQ, CH_2Cl_2:H_2O (20:1), 84%; b. i. $(Me_3SiCH_2CH_2O)_2PNEt_2$ **150**, 1*H*-tetrazole, THF; ii. MCPBA, CH_2Cl_2, -60°C to room temperature, 85%; c. NH_3 sat. MeOH, 73%; d. 48-51% HF:MeCN:H_2O (5:85:15); e. 1 M NaOH; f. Ion-exchange chromatography, 53%.

Deprotection of the protected alcohol **158** by stirring in a solution of hydrofluoric acid followed by conversion to the sodium salt led to a mixture of the desired fully deprotected phosphate **83b** and the phosphate with one 2-(trimethylsilyl)ethyl group still attached **159**. Purification of this mixture by ion-exchange chromatography gave the desired 5-phosphate **83b** in 53% yield.

The desired 5-phosphate **83b** was also prepared by conversion to the di(cyclohexylammonium) salt as opposed to the disodium salt in approximately 50% yield.

3.4 Summary of Chapter 3

The syntheses of the 1-phosphate **152** and the 5-phosphate **83b** have been successfully achieved from the synthetically useful cyclopentenone **61**. This compound was prepared either from D-ribose **126** as outlined in Scheme 3.4 or from cyclopentadiene **36** as shown in Scheme 2.12.

Unfortunately due to time constraints the synthesis of the pyrophosphorylated intermediate **84b** and feeding studies with these compounds could not be carried out.

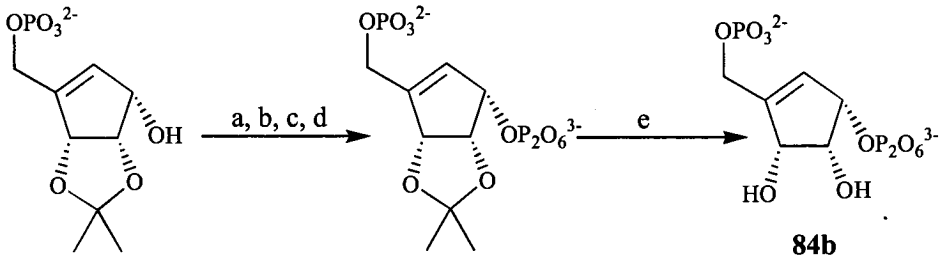
3.5 Future Work

3.5.1 Preparation of pyrophosphorylated intermediate 84b

Two approaches to the synthesis of the pyrophosphorylated intermediate **84b** could be examined.

(a) Synthetic approach

The pyrophosphorylation of the 5-phosphate **83b** could be investigated using the conditions shown in Figure 3.3, which have been reported for the preparation of the saturated analogue of the pyrophosphate in which the double bond is absent.[112,113]



Reagents and conditions: a. bis(morpholino)-2-cyanoethylphosphite, 1*H*-tetrazole, THF; b. MCPBA; c. i. NH_3, MeOH; ii. Amberlite IR 120, sodium form; d. $(n\text{-}Bu)_3NH^+$ $H_2PO_4^-$, C_5H_5N; e. H^+, H_2O.

Figure 3.3 Proposed synthesis of pyrophosphate **84b**

(b) Enzymatic approach

The alternative approach to the synthesis of the pyrophosphate **84b** could be based on the use of an enzyme-catalysed pyrophosphorylation procedure as shown in Figure 3.4.[114]

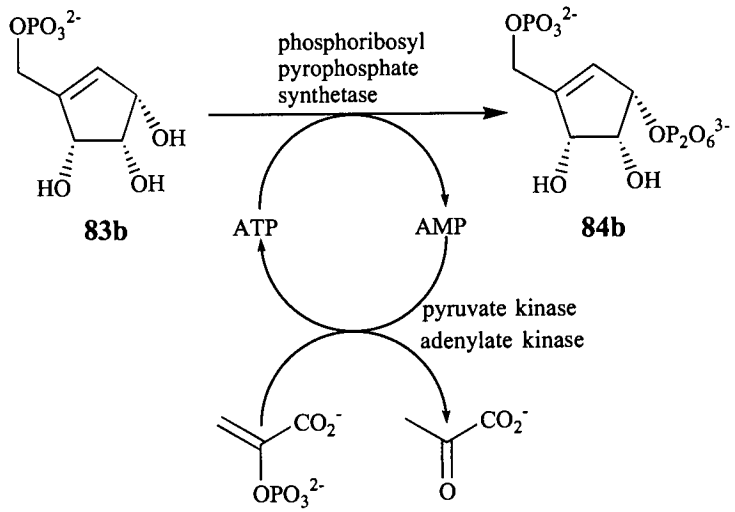


Figure 3.4 Proposed enzymatic synthesis of pyrophosphate **84b**

This approach to the synthesis of the pyrophosphate **84b** has the advantage that the mild conditions are commensurate with the anticipated instability of the pyrophosphate **84b**. However, studies carried out by Parry *et al.*[107] found that the 5-phosphate **83b** was an inhibitor, rather than a substrate for 5-phosphoribosyl α-1-pyrophosphate synthetases of both bacterial and human origin. This result suggests that the preparation of the pyrophosphate **84b** *via* this procedure may be difficult.

3.5.2 Feeding studies

The phosphorylated compounds cannot pass through the cell wall. Therefore the feeding experiments cannot be carried out by simply feeding these substrates to the CC940 mutant of *Streptomyces citricolor* grown on GAM 6:6 medium, as described for the tetrol and keto-tetrol compounds in Section 2.4.

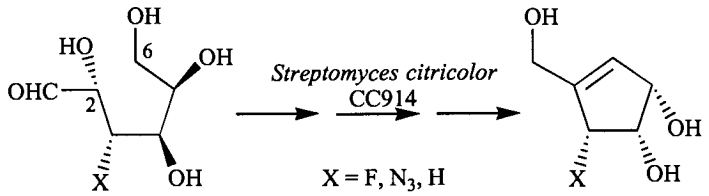
Therefore, in order to establish that the phosphate **83b** and the pyrophosphate **84b** are intermediates on the biosynthetic pathway, cell-free extracts of *Streptomyces*

citricolor will be required. These cell-free extracts could be obtained by adapting a procedure developed by Parry *et al.*[87] to obtain a cell-free enzyme extract that is able to convert tetrol **83a** to neplanocin A and aristeromycin. Using this cell-free enzyme extract the phosphate **83b** and the pyrophosphate **84b** will be assessed as intermediates on the biosynthetic pathway.

4 Future Work – Exploitation of the Biosynthetic Pathway for the Preparation of Novel Carbocyclic Compounds

Many further investigations must be carried out in order to obtain a complete understanding of the biosynthesis of aristeromycin and neplanocin A from D-glucose by the organism *Streptomyces citricolor*. However, an important consequence of understanding the biosynthetic pathway in detail is that it should be possible to exploit the enzymes involved for the conversion of unnatural substrates thereby generating novel chiral products and unnatural carbocyclic nucleoside analogues that would be difficult to prepare by alternative chemical procedures. These investigations would include:

i. feeding analogues of D-glucose in which the 3-OH group is replaced by F, N_3, H *etc*. For these experiments, in addition to the wild-type strain, a mutant of *Streptomyces citricolor* could be used. For example, the secretor mutant CC914 which produces tetrol **83a** when supplied with glucose, would yield the corresponding analogues of tetrol **83a** as shown in Scheme 4.1.



Scheme 4.1

ii. synthesising analogues of the keto-tetrol **95**, enone **82a** and tetrol **83a** and then feeding these to the converter mutant CC940 to obtain modified carbocyclic structures and novel carbocyclic nucleoside analogues.

iii. replacing adenine with alternative purine precursors to obtain novel analogues of aristeromycin and neplanocin A.

5 Experimental

5.1 General Experimental

5.1.1 Instrumentation

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on either a Varian Gemini 200, Bruker AC 250, Bruker WH 360 or Varian UNITY INOVA 600 instrument. Chemical shifts (δ_H, δ_C, δ_P) are reported in parts per million (ppm) and coupling constants (J) are reported in Hertz (Hz) and quoted to the nearest 0.5 Hz.

Electron Impact (EI) mass spectrometry was carried out on a Finnegan 4500 or a Finnegan 4600 instrument and Fast Atom Bombardment (FAB) mass spectrometry was performed using a Kratos MS50TC instrument. Electrospray (ES) and Atmospheric Pressure Chemical Ionisation (APCI) mass spectrometry was carried out on a Micromass Platform II instrument.

Infrared spectra were recorded on a Biorad FTS-7 or a Perkin Elmer Paragon 1000 FT-IR spectrometer with the frequencies (ν) being measured in wavenumbers (cm^{-1}). Samples were recorded as thin films using either sodium chloride plates or disposable IR cards (3M, type 61, polyethylene 19 mm aperture), as nujol mulls or in solution.

Optical rotations were measured on an Optical Activity AA-1000 polarimeter with a cell path length of 1 dm and concentrations (c) quoted in g/100 ml (sodium 589 nm detection). $[\alpha]_D^T$ values are given in 10^{-1} deg cm^2 g^{-1}.

Elemental analysis was performed using a Perkin-Elmer 2400 CHN Elemental Analyser.

Melting points were obtained on a Gallenkamp melting point apparatus and are given in (°C) uncorrected.

Chiral gas chromatography was carried out on a Shimadzu GC-17A Gas Chromatograph with Shimadzu C-R6A Chromatopac recorder. A Supelco β-dex 120 column (30 m x 0.25 mm) was used with a column temperature of 125°C, injector temperature of 260°C, detector temperature of 300°C and helium as the carrier gas. Retention times (R_t) are given in minutes.

5.1.2 Chromatography

Analytical thin layer chromatography (TLC) was carried out on Merck aluminium backed plates coated with silica gel 60 F_{254} and components were identified using *p*-anisaldehyde, ammonium molybdate and potassium permanganate dips.

Flash column chromatography was carried out using the appropriately sized parallel-sided column filled with silica gel 60 (Merck 9385, particle size 0.04-0.063 mm).

Phosphate esters were purified by ion-exchange chromatography using the following procedure:

1 M triethylammonium bicarbonate buffer solution was prepared by bubbling carbon dioxide through an immiscible mixture of freshly distilled triethylamine (139 ml) and deionized water (861 ml) until the triethylamine had dissolved and a pH of 8 was obtained.

AG 1-X2 resin (Biotechnology grade, 200-400 mesh, hydroxide form, strongly basic anion exchange resin, approximately 0.5 g), made into a slurry with deionized water, was pipetted into a small parallel-sided column (diameter = 8 mm) to give a resin bed of approximately 6 cm. A piece of cotton-wool was placed on top and the resin was then washed with deionized water before being converted to the bicarbonate form by washing with 1 M triethylammonium bicarbonate buffer solution (final pH = 8). When converted the resin was washed repeatedly with deionized water (final pH = 6).

The mixture containing the phosphate ester (approximately 20 mg) was dissolved in the minimum amount of deionized water and the resulting solution applied to the top of the resin bed. The phosphates were then eluted with portions (20 ml) of increasing concentration of triethylammonium bicarbonate buffer solution, starting with water then increasing by 5 mM to 50 mM.

5.1.3 Solvents and reagents

All solvents and reagents were used as supplied from commercial sources unless otherwise stated.

Where a solvent has been described as anhydrous, it was either purchased as anhydrous grade or was distilled prior to use. Dichloromethane was distilled from calcium hydride and tetrahydrofuran was distilled from sodium benzophenone ketyl. DBU, diethylamine, diisopropylamine, dimethyl methylphosphonate, phosphorous trichloride, trichloroacetonitrile and triethylamine were distilled before use.

Petroleum ether refers to the fraction bp 45-60°C.

Novozyme® was received as a gift from Novo-Nordisk.

n-BuLi was titrated against *N*-pivaloyl-*o*-toluidine[115] or diphenylacetic acid before use. Sodium hydride (60% dispersion in mineral oil) was washed under an atmosphere of nitrogen with anhydrous petroleum ether before use.

Amberlite IR 120 (H) ion-exchange resin was activated by stirring in 2 M HCl for 30 min then washing with water until the washings were neutral pH.

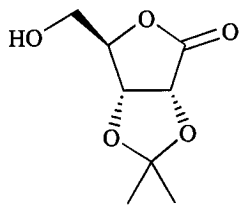
Cyclopentadiene was obtained by heating dicyclopentadiene in distillation apparatus equipped with a 30 cm vigreux column. The cyclopentadiene was distilled at 40-44°C at a rate of approximately 0.5 g/min, and collected in a receiver flask cooled in an ice bath. Excessive heating was avoided in order to minimise distillation of the dimer.

Jones reagent (1.34 M) was prepared by dissolving chromium trioxide (13.40 g, 0.13 mol) in concentrated sulfuric acid (12 ml) and then diluting to 100 ml with distilled water.[93]

5.2 Experimental Procedures for Chapter 2

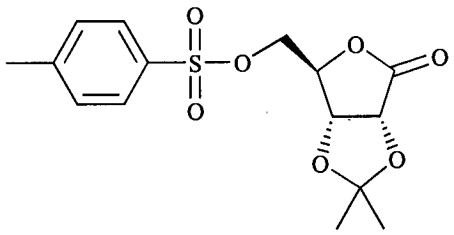
5.2.1 Synthesis of *ent*-keto-tetrol *ent*-95

(a) 2,3-*O*-Isopropylidene-D-ribonolactone *ent*-103



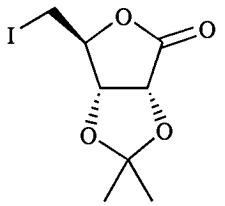
A solution of D-(+)-ribonic acid-γ-lactone (10.32 g, 69.7 mmol, 1.0 eq) and *p*-toluenesulfonic acid (1.05 g, 5.52 mmol, 0.08 eq) in acetone (400 ml) was stirred at room temperature under nitrogen for 18 h. Sodium bicarbonate was then added until a pH = 6 was obtained and the mixture was stirred for 1 h before being filtered through a pad of silica. The silica was washed with ethyl acetate (500 ml) and the filtrate was concentrated to give the *protected ribonolactone* ***ent*-103** as a white solid (12.98 g, 99%); mp 134-138°C (lit.[116] mp 134-137°C); (Found: C, 51.19; H, 6.71. $C_8H_{12}O_5$ requires C, 51.06; H, 6.43%); R_f 0.74 (EtOAc); $[\alpha]_D^{24}$ –69.9 (*c* 0.81 in $CHCl_3$); ν_{max}(nujol)/cm^{-1} 3463 (OH), 1764 (C=O), 1383 (CMe_2), 1224 (CO); δ_H(250 MHz; $CDCl_3$) 4.79 (2H, 2d, *J* 5.5 and 5.5, 2-H and 3-H), 4.62 (1H, t, *J* 2.0, 4-H), 3.98 (1H, br d, *J* 12.0, CH_2), 3.77 (1H, br d, *J* 12.0, CH_2), 2.84 (1H, br s, OH), 1.46 (3H, s, CCH_3), 1.36 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 175.2 (C=O), 113.1 (CMe_2), 82.9 (CH), 78.3 (CH), 75.6 (CH), 61.8 (CH_2), 26.7 (CH_3), 25.4 (CH_3); *m/z* (FAB) 189 (MH^+, 77%), 377 (MH^+ dimer, 21); [Found: MH^+, 189.07581. $C_8H_{12}O_5$ requires MH, 189.07630].

(b) 5-*O*-(*p*-Toluenesufonyl)-2,3-*O*-isopropylidene-D-ribonolactone *ent*-104



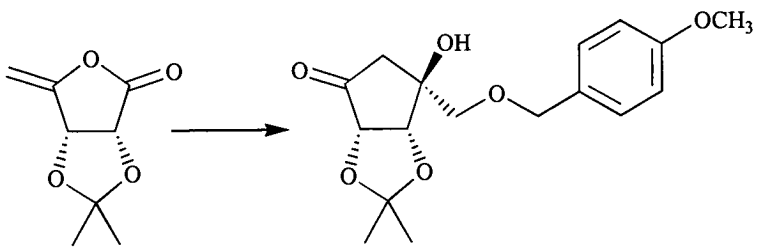
A mixture of the protected ribonolactone ***ent*-103** (12.90 g, 68.6 mmol, 1.0 eq), *p*-toluenesulfonyl chloride (23.45 g, 0.12 mol, 1.8 eq) and pyridine (15.00 ml, 0.19 mol, 2.7 eq) in chloroform (100 ml) was stirred at room temperature under nitrogen for 19 h. Water (15 ml) was added and the mixture was stirred for a further 30 min. The mixture was diluted with dichloromethane (250 ml) and then poured over 1 M citric acid (2 x 250 ml). The organic portion was washed with brine (300 ml), dried over sodium sulfate, filtered and concentrated. Diethyl ether (100 ml) was added to the resulting yellow oil and after slight swirling a white solid crystallised out. The solid was collected by filtration to give the *tosylate* ***ent*-104** as an off-white solid (18.72 g, 80%); mp 117°C (lit.[117] mp 117.5-118°C); (Found: C, 52.64; H, 5.46. $C_{15}H_{18}O_7S$ requires C, 52.62; H, 5.30%); R_f 0.56 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ –15.2 (*c* 1.05 in $CHCl_3$); ν_{max}(nujol)/cm^{-1} 1784 (C=O), 1594 (C=C_{ar}), 1373 (CMe_2), 1215 (CO), 1175 (SO_2-O); δ_H(200 MHz; $CDCl_3$) 7.74 (2H, d, *J* 8.5, 2 x H_{ar}), 7.37 (2H, d, *J* 8.5, 2 x H_{ar}), 4.75 (2H, 2d, *J* 5.5 and 5.5, 2-H and 3-H), 4.67 (1H, t, *J* 2.0, 4-H), 4.33 (1H, dd, *J* 2.0 and 11.0, CH_2), 4.16 (1H, dd, *J* 2.0 and 11.0, CH_2), 2.45 (3H, s, $PhCH_3$), 1.44 (3H, s, CCH_3), 1.37 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 173.1 (C=O), 145.9 (C_{ar}), 131.5 (C_{ar}), 130.2 (2 x CH_{ar}), 127.9 (2 x CH_{ar}), 113.8 (CMe_2), 79.0 (CH), 77.3 (CH), 75.0 (CH), 68.2 (CH_2), 26.6 (CH_3), 25.5 (CH_3), 21.7 ($PhCH_3$); *m/z* (FAB) 343 (MH^+, 19%), 155 ($CH_3C_6H_4SO_2$, 8), 91 ($CH_3C_6H_4$, 100); [Found: MH^+, 343.08544. $C_{15}H_{18}O_7S$ requires MH, 343.08515].

(c) 5-Iodo-5-deoxy-2,3-*O*-isopropylidene-D-ribonolactone ent-105[89]



A stirred solution of the tosylate ***ent*-104** (17.74 g, 51.8 mmol, 1.0 eq) and sodium iodide (15.54g, 0.10 mol, 2.0 eq) in acetone (250 ml) was heated at reflux for 18 h before being allowed to cool to room temperature. The mixture was then filtered through Celite and the filtrate was concentrated under reduced pressure. The concentrated residue was dissolved in ethyl acetate (300 ml) and the solution was washed with saturated aqueous sodium thiosulfate (250 ml). The organic portion was dried over sodium sulfate, filtered and concentrated to give a pale yellow solid. Recrystallisation of the solid from ethyl acetate-hexane gave the *iodide* ***ent*-105** as white needle-like crystals (13.14 g, 85%); mp 93-94°C (lit.[89] mp 95-97°C); (Found: C, 32.32; H, 3.75. $C_8H_{11}IO_4$ requires C, 32.24; H, 3.72%); R_f 0.36 (EtOAc:hexane, 1:3); $[\alpha]_D^{24}$ –35.1 (*c* 1.10 in $CHCl_3$); ν_{max}(nujol)/cm^{-1} 1778 (C=O), 1371 (CMe_2), 1216 (CO); δ_H(250 MHz; $CDCl_3$) 4.97 (1H, d, *J* 6.0, 2-H), 4.63 (1H, m, 4-H), 4.60 (1H, d, *J* 6.0, 3-H), 3.40 (2H, m, CH_2), 1.46 (3H, s, CCH_3), 1.38 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 172.9 (C=O), 113.9 (CMe_2), 80.7 (CH), 80.2 (CH), 75.1 (CH), 26.4 (CH_3), 25.3 (CH_3), 5.5 (CH_2); *m/z* (FAB) 299 (MH^+, 41%); [Found: MH^+, 298.97853. $C_8H_{11}IO_4$ requires MH, 298.97804].

(d) (2*S*,3*R*,4*S*)-2,3-(Isopropylidenedioxy)-4-[(*p*-methoxybenzyloxy)methyl]-4-hydroxy-cyclopentan-1-one *ent*-106

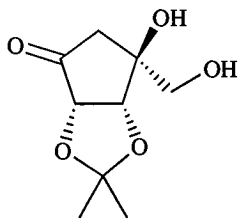


DBU (1.02 ml, 6.84 mmol, 1.0 eq) was added dropwise to a solution of the iodide ***ent*-105** (2.04 g, 6.84 mmol, 1.0 eq) in anhydrous THF (15 ml) to give a pale yellow solution containing a white solid, which was stirred at room temperature under nitrogen for 40 min. The mixture was cooled to -78°C and the solution was then separated from the solid by filter cannulation of the solution into a round-bottomed flask under nitrogen. The resulting pale brown solution of *enol-lactone* ***ent*-99** was then cooled to -78°C.

A solution of [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** (3.07 g, 6.96 mmol, 1.02 eq) in anhydrous THF (29 ml) was cooled to -78°C. *n*-BuLi (1.57 M solution in hexanes, 4.36 ml, 6.84 mmol, 1.0 eq) was then added dropwise over 2 min to give a pale yellow solution which was stirred at -78°C for 5 min. The cooled enol-lactone ***ent*-99** solution was then added quickly *via* nitrogen pressure transfer through a double tipped needle. The resulting yellow solution was stirred at -78°C for 17 min after which time the reaction mixture was quenched by the addition of saturated aqueous ammonium chloride (20 ml). The mixture was then allowed to warm to room temperature before being poured into ethyl acetate (20 ml) and saturated aqueous ammonium chloride (10 ml). The organic layer was separated and the aqueous portion was extracted with ethyl acetate (3 x 75 ml). The combined organic portions were washed with water (150 ml) and brine (150 ml), dried over sodium sulfate, filtered and concentrated. The resulting colourless liquid containing a yellow oil was purified by flash column chromatography using ethyl acetate-hexane (3:7) as the eluent to give the *PMB-protected β-hydroxycyclopentanone* ***ent*-106** as a pale yellow oil (762 mg, 35%); R_f 0.49 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ +97.2 (*c* 1.04 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3460 (OH), 2988, 2936, 2866 and 2838 (CH), 1760 (C=O), 1612 (C=C_{ar}), 1514 (C=C_{ar}), 1374 (CMe_2), 1248 (CO); δ_H(250 MHz; $CDCl_3$) 7.26 (2H, d, *J* 8.5, 2 x H_{ar}), 6.89 (2H, d, *J* 8.5, 2 x H_{ar}), 4.61-4.38 (4H, m, $PhCH_2$, 2-H and 3-H), 3.81 (3H, s, OCH_3), 3.75 (1H, d, *J* 9.5, CH_2O), 3.55 (1H, d, *J* 9.5, CH_2O), 2.91 (1H, br s, OH), 2.54 (1H, d, *J* 18.0, C-6-H_{2endo}), 2.24 (1H, dt, *J* 1.5 and 18.0, C-6-H_{2exo}), 1.39 (3H, s, CCH_3), 1.35 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 211.0 (C=O), 159.3 (C_{ar}), 129.5 (C_{ar}), 129.3 (2 x CH_{ar}), 113.8 (2 x CH_{ar}), 113.1 (CMe_2), 81.2 (CH), 79.3 (CH), 75.0 (C-4), 73.2 (CH_2), 71.0 (CH_2), 55.2 (OCH_3), 43.5 (C-$6H_2$), 26.8

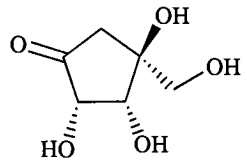
(CH_3), 24.9 (CH_3); *m/z* (EI) 322 (M^+, 1%), 201 (M-$CH_3OC_6H_4CH_2$, 3), 137 ($CH_3OC_6H_4CH_2O$, 19), 121 ($CH_3OC_6H_4CH_2$, 100); [Found: M^+, 322.14186. $C_{17}H_{22}O_6$ requires M, 322.14164].

(e) (2*S*,3*R*,4*S*)-2,3-(Isopropylidenedioxy)-4-hydroxymethyl-4-hydroxy-cyclopentan-1-one *ent*-107



Palladium on activated charcoal (10% Pd, 198 mg, 0.018 g of Pd, 0.186 mmol, 0.5 eq) was added to a solution of the PMB-protected β-hydroxycyclopentanone ***ent*-106** (120 mg, 0.372 mmol, 1.0 eq) in anhydrous THF (4 ml) and the mixture was vigorously stirred for 3 h under an atmosphere of hydrogen. The catalyst was then removed by filtering through Celite. The Celite was washed repeatedly with ethyl acetate and the filtrate was concentrated under reduced pressure. The resulting pale yellow oil was purified by flash column chromatography using ethyl acetate-hexane (4:1) as the eluent to give the *acetonide-protected β-hydroxycyclopentanone* ***ent*-107** as a colourless oil (51 mg, 68%); R_f 0.49 (EtOAc); $[\alpha]_D^{22}$ +171.1 (*c* 1.03 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3437 (OH), 2988, 2938 and 2879 (CH), 1760 (C=O), 1376 (CMe_2), 1213 (CO); δ_H(250 MHz; $CDCl_3$) 4.55 (1H, dd, *J* 1.5 and 5.0, 3-H), 4.43 (1H, ddd, *J* 1.0, 1.5 and 5.0, 2-H), 3.93 (1H, d, *J* 11.0, CH_2OH), 3.74 (1H, d, *J* 11.0, CH_2OH), 3.28 (1H, br s, C-4-OH), 2.63 (1H, dd, *J* 1.0 and 18.0, C-6-H_{2endo}), 2.25 (1H, dt, *J* 1.5 and 18.0, C-6-H_{2exo}), 1.41 (3H, s, CCH_3), 1.34 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 211.0 (C=O), 113.4 (CMe_2), 81.1 (CH), 79.3 (CH), 75.9 (C-4), 64.5 (CH_2OH), 43.2 (C-$6H_2$), 26.7 (CH_3), 24.8 (CH_3); *m/z* (FAB) 203 (MH^+, 100%), 185 (M-OH, 6), 171 (M-CH_2OH, 2); [Found: MH^+, 203.09205. $C_9H_{14}O_5$ requires MH, 203.09195].

(f) (2*S*,3*R*,4*S*)-4-Hydroxymethyl-2,3,4-trihydroxy-cyclopentan-1-one *ent*-95



Trifluoroacetic acid (0.5 ml) was added to a solution of the protected β-hydroxycyclopentanone ***ent*-107** (108 mg, 0.534 mmol, 1.0 eq) in anhydrous dichloromethane (2 ml). The resulting pale yellow solution was stirred at room temperature for 3 min before being concentrated under reduced pressure to give a brown oil. The brown oil was purified by flash column chromatography using methanol-chloroform (2:8) as the eluent to give the desired *keto-tetrol* ***ent*-95** as a colourless foam (23 mg, 26%). ^{1}H, ^{13}C NMR and mass spectrometry showed that the keto-tetrol ***ent*-95** was contaminated with minor traces of the eliminated enone ***ent*-82a** (47:1), determined by 250 MHz ^{1}H NMR analysis.

(2S,3R,4S)-4-Hydroxymethyl-2,3,4-trihydroxy-cyclopentan-1-one ***ent*-95**

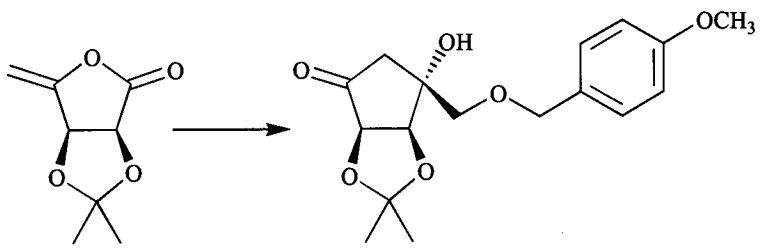
R_f 0.38 ($MeOH:CHCl_3$, 3:7); δ_H(250 MHz; D_2O) 4.66 (1H, d, *J* 4.5, 2-H), 4.15 (1H, d, *J* 4.5, 3-H), 3.77 (1H, d, *J* 12.0, C*H*$_2$OH), 3.65 (1H, d, *J* 12.0, C*H*$_2$OH), 2.34 (2H, s, C-6-H_2); δ_C(63 MHz; D_2O) 215.4 (C=O), 73.9 (CH), 73.6 (C-4), 70.7 (CH), 61.5 (CH_2OH), 39.6 (C-6H_2); *m/z* (FAB) 163 (MH^+, 2%), 131 (M-CH_2OH, 60).

(2S,3S)-4-Hydroxymethyl-2,3-dihydroxy-cyclopent-4-en-1-one ***ent*-82a**

R_f 0.44 ($MeOH:CHCl_3$, 3:7); *m/z* (FAB) 145 (MH^+, 3%), 113 (M-CH_2OH, 5). Peaks in ^{1}H and ^{13}C NMR spectra were too small to measure.

5.2.2 Synthesis of keto-tetrol 95

(a) (2*R*,3*S*,4*R*)-2,3-(Isopropylidenedioxy)-4-[(*p*-methoxybenzyloxy)methyl]-4-hydroxy-cyclopentan-1-one 106

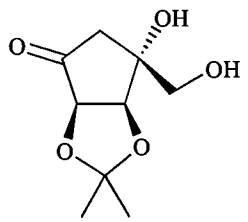


DBU (1.02 ml, 6.84 mmol, 1.0 eq) was added dropwise to a solution of the iodide **105**, prepared from L-ribose by I. V. J. Archer (2.04 g, 6.84 mmol, 1.0 eq) in anhydrous THF (15 ml) to give a pale yellow solution containing a white solid, which was stirred at room temperature under nitrogen for 40 min. The mixture was then cooled to -78°C and the solution was separated from the solid by filter cannulation of the solution into a round-bottomed flask under nitrogen. The resulting pale brown solution of *enol-lactone* **99** was then cooled to -78°C.

A solution of [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** (3.07 g, 6.96 mmol, 1.02 eq) in anhydrous THF (29 ml) was cooled to -78°C. *n*-BuLi (1.53 M solution in hexanes, 4.47 ml, 6.84 mmol, 1.0 eq) was then added dropwise over 2 min to give a pale yellow solution which was stirred at -78°C for 5 min. The cooled enol-lactone **99** solution was then added quickly *via* nitrogen pressure transfer through a double tipped needle and the resulting yellow solution was stirred at -78°C for 15 min after which time the reaction mixture was quenched by the addition of saturated aqueous ammonium chloride (15 ml). The mixture was allowed to warm to room temperature before being poured into ethyl acetate (20 ml) and saturated aqueous ammonium chloride (10 ml). The organic layer was separated and the aqueous portion was extracted with ethyl acetate (2 x 100 ml). The combined organic portions were washed with water (175 ml) and brine (175 ml), dried over sodium sulfate, filtered and concentrated. The resulting colourless liquid containing a yellow oil was purified by flash column chromatography using ethyl acetate-

hexane (7:13) as the eluent to give the *PMB-protected β-hydroxycyclopentanone* **106** as a pale yellow oil (1.21 g, 55%); R_f 0.55 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ –92.0 (*c* 0.97 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3464 (OH), 2989, 2937 and 2866 (CH), 1761 (C=O), 1613 (C=C_{ar}), 1514 (C=C_{ar}), 1376 (CMe_2), 1250 (CO); δ_H(250 MHz; $CDCl_3$) 7.26 (2H, d, *J* 9.0, 2 x H_{ar}), 6.89 (2H, d, *J* 9.0, 2 x H_{ar}), 4.61-4.37 (4H, m, $PhCH_2$, 2-H and 3-H), 3.80 (3H, s, OCH_3), 3.75 (1H, d, *J* 9.5, CH_2O), 3.55 (1H, d, *J* 9.5, CH_2O), 2.93 (1H, br s, OH), 2.54 (1H, d, *J* 18.0, C-6-H_{2exo}), 2.24 (1H, dt, *J* 1.5 and 18.0, C-6-H_{2endo}), 1.39 (3H, s, CCH_3), 1.34 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 211.0 (C=O), 159.3 (C_{ar}), 129.5 (C_{ar}), 129.3 (2 x CH_{ar}), 113.8 (2 x CH_{ar}), 113.1 (CMe_2), 81.2 (CH), 79.3 (CH), 75.0 (C-4), 73.2 (CH_2), 71.0 (CH_2), 55.2 (OCH_3), 43.5 (C-6H_2), 26.8 (CH_3), 24.9 (CH_3); *m/z* (EI) 322 (M^+, 1%), 201 (M-$CH_3OC_6H_4CH_2$, 3), 137 ($CH_3OC_6H_4CH_2O$, 12), 121 ($CH_3OC_6H_4CH_2$, 100); [Found: M^+, 322.14213. $C_{17}H_{22}O_6$ requires M, 322.14164].

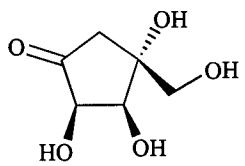
(b) (2*R*,3*S*,4*R*)-2,3-(Isopropylidenedioxy)-4-hydroxymethyl-4-hydroxy-cyclopentan-1-one 107



Palladium on activated charcoal (10% Pd, 867 mg, 0.087 g of Pd, 0.814 mmol, 0.5 eq) was added to a stirred solution of the PMB-protected β-hydroxycyclopentanone **106** (525 mg, 1.63 mmol, 1.0 eq) in anhydrous THF (16 ml). The mixture was then vigorously stirred under an atmosphere of hydrogen for 2 h 20 min. The mixture was filtered through Celite, the Celite was washed repeatedly with ethyl acetate, and the filtrate was concentrated under reduced pressure. The resulting pale yellow oil was purified by flash column chromatography using ethyl acetate-hexane (4:1) as the eluent to give the *acetonide-protected β-hydroxycyclopentanone* **107** as a colourless oil (188 mg, 57%); R_f 0.53 (EtOAc); $[\alpha]_D^{24}$ –163.1 (*c* 1.17 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3437 (OH), 2989, 2938 and 2879 (CH), 1760 (C=O), 1378 (CMe_2), 1213 (CO); δ_H(250 MHz; $CDCl_3$) 4.55 (1H, dd, *J* 1.5 and 5.0, 3-H), 4.43 (1H, ddd, *J* 1.0, 1.5 and

5.0, 2-H), 3.93 (1H, d, *J* 11.0, C*H*$_2$OH), 3.73 (1H, d, *J* 11.0, C*H*$_2$OH), 3.14 (1H, br s, C-4-OH), 2.62 (1H, dd, *J* 1.0 and 18.0, C-6-H$_{2exo}$), 2.48 (1H, br s, OH), 2.25 (1H, dt, *J* 1.5 and 18.0, C-6-H$_{2endo}$), 1.41 (3H, s, CCH$_3$), 1.35 (3H, s, CCH$_3$); δ_C(63 MHz; $CDCl_3$) 210.8 (C=O), 113.4 (CMe_2), 81.2 (CH), 79.3 (CH), 75.8 (C-4), 64.5 (CH_2OH), 43.1 (C-6H_2), 26.7 (CH_3), 24.8 (CH_3); *m/z* (FAB) 203 (MH^+, 100%), 185 (M-OH, 2), 171 (M-CH_2OH, 4); [Found: MH^+, 203.09265. $C_9H_{14}O_5$ requires MH, 203.09195].

(c) (2*R*,3*S*,4*R*)-4-Hydroxymethyl-2,3,4-trihydroxy-cyclopentan-1-one 95



Trifluoroacetic acid (0.7 ml) was added to a solution of the protected β-hydroxycyclopentanone **107** (150 mg, 0.742 mmol, 1.0 eq) in anhydrous dichloromethane (2.8 ml). The resulting pale yellow solution was stirred at room temperature for 3 min before being concentrated under reduced pressure to give a brown oil. The brown oil was purified by flash column chromatography using methanol-chloroform (1:4) as the eluent to give the desired *keto-tetrol* **95** as a colourless oil (48 mg, 40%). ^1H, ^{13}C NMR and mass spectrometry showed that the keto-tetrol **95** was contaminated with minor traces of the eliminated enone **82a** (10:1), determined by 600 MHz ^1H NMR analysis.

(2R,3S,4R)-4-Hydroxymethyl-2,3,4-trihydroxy-cyclopentan-1-one **95**

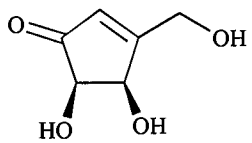
R_f 0.32 ($MeOH:CHCl_3$, 3:7); δ_H(600 MHz; D_2O) 4.74 (1H, d, *J* 4.5, 2-H), 4.24 (1H, d, *J* 4.5, 3-H), 3.86 (1H, d, *J* 12.0, C*H*$_2$OH), 3.73 (1H, d, *J* 12.0, C*H*$_2$OH), 2.43 (2H, s, C-6-H_2); δ_C(91 MHz; D_2O) 218.4 (C=O), 76.9 (CH), 76.6 (C-4), 73.6 (CH), 64.5 (CH_2OH), 42.6 (C-6H_2); *m/z* (FAB) 163 (MH^+, 2%), 131 (M-CH_2OH, 77).

(2R,3R)-4-Hydroxymethyl-2,3-dihydroxy-cyclopent-4-en-1-one **82a**

R_f 0.39 ($MeOH:CHCl_3$, 3:7); δ_H(600 MHz; D_2O) 6.33 (1H, t, *J* 2.0, =CH), 4.79 (1H, obscured by D_2O residual peak, 2-H), 4.68 (1H, dd, *J* 2.0 and 19.0, C*H*$_2$OH), 4.54

(1H, dd, *J* 2.0 and 19.0, C*H*$_2$OH), 4.34 (1H, d, *J* 6.0, 3-H); δ_C(91 MHz; D_2O) 209.6 (C=O), 179.6 (C-4), 126.5 (=CH), 72.5 (CH), 69.0 (CH), 60.5 (CH_2OH); *m/z* (FAB) 145 (MH^+, 3%), 113 (M-CH_2OH, 5).

(d) (2*R*,3*R*)-4-Hydroxymethyl-2,3-dihydroxy-cyclopent-4-en-1-one 82a



Trifluoroacetic acid (1 ml) was added to a stirred solution of the keto-tetrol **95** and enone **82a** (30 mg) in dichloromethane (2 ml) and methanol (0.5 ml). The resulting yellow solution was stirred at room temperature for 1 h after which time further TFA (1 ml) was added and the solution was stirred for a further 3 h 45 min. TLC showed that the keto-tetrol was still present therefore additional TFA (1 ml) was added and the solution was stirred at room temperature for 18 h. The brown solution was then concentrated under reduced pressure and the resulting brown oil was purified by flash column chromatography using methanol-chloroform (1:4) as the eluent to give the *enone* **82a** as a yellow oil (12 mg, ~45%); R_f 0.39 (MeOH:$CHCl_3$, 3:7); ν_{max}(neat)/cm^{-1} 3368 (OH), 2989, 2921 and 2853 (CH), 1755 (C=O), 1682 (C=C), 1212 (CO); δ_H(600 MHz; D_2O) 6.28 (1H, s, =CH), 4.80 (1H, obscured by D_2O residual peak, 2-H), 4.63 (1H, d, *J* 19.0, C*H*$_2$OH), 4.49 (1H, d, *J* 19.0, C*H*$_2$OH), 4.29 (1H, d, *J* 5.5, 3-H); δ_C(91 MHz; D_2O) 209.6 (C=O), 179.6 (C-4), 126.5 (=CH), 72.5 (CH), 68.9 (CH), 60.5 (CH_2OH); *m/z* (FAB) 145 (MH^+, 4%), 113 (M-CH_2OH, 10); [Found: MH^+, 145.05101. $C_6H_8O_4$ requires MH, 145.05008].

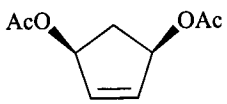
5.2.3 Synthesis of keto-tetrol 116

(a) Cyclopentadiene monoepoxide 121[51]

Sodium acetate (12.00 g) was added to peracetic acid (36-40% wt. solution in acetic acid, 196 ml, 1.05 mol, 0.72 eq) and the mixture was set aside until dissolution was complete.

Cyclopentadiene **36** (96.12 g, 1.45 mol, 1.0 eq) was added to a mechanically stirred mixture of sodium carbonate (483 g) in dichloromethane (820 ml) cooled to 0°C. The buffered peracetic acid solution was then added dropwise *via* a dropping funnel. After approximately 10 min carbon dioxide gas was given off and the rate of addition was controlled by monitoring the rate of carbon dioxide passing through a bubbler. After 1 h, when the addition was complete, the cooling bath was removed and the mixture was stirred at room temperature for 1 h 30 min. A sample was removed and dropped into saturated aqueous sodium iodide (brown colour in presence of oxidant) no colour was observed therefore the reaction was deemed to be complete. The mixture was filtered and the filtrate concentrated under slight vacuum. Distillation at 160 mmHg (64°C) (lit.[118] bp 39-41°C at 46 mm) gave the *cyclopentadiene monoepoxide* **121** as a yellow liquid (28.13 g, 24% based on cyclopentadiene); ν_{max}(neat)/cm^{-1} 3050 (epoxide CH), 2910 (CH), 1673 (C=C), 1283 (CO), 913 and 813 (epoxide CO); δ_H(200 MHz; $CDCl_3$) 6.13 (1H, m, =CH), 6.00 (1H, m, =CH), 3.90 (1H, m, CHO), 3.80 (1H, m, CHO), 2.67-2.31 (2H, m, CH_2); δ_C(63 MHz; $CDCl_3$) 137.7 (=CH), 131.1 (=CH), 59.1 (CHO), 56.7 (CHO), 35.4 (CH_2); *m/z* (FAB) 83 (MH^+, 100%), 66 (M-O, 34); [Found (EI): M^+, 82.04156. C_5H_6O requires M, 82.04186].

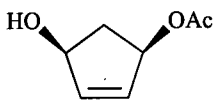
(b) *cis*-1,4-Diacetoxycyclopent-2-ene 48[51]



A solution of cyclopentadiene monoepoxide **121** (25.00 g, 0.31 mol, 1.0 eq) in anhydrous THF (50 ml) was added dropwise over 10 min to an ice-cooled, stirred solution of tetrakis(triphenylphosphine)palladium(0) (1.76 g, 1.53 mmol, 0.5 mol %) and acetic anhydride (30 ml, 0.32 mol, 1.04 eq) in anhydrous THF (250 ml). After stirring at 0°C for 10 min the mixture was filtered through silica, the silica was

washed with diethyl ether (200 ml) and the filtrate was concentrated. The resulting brown oil was purified by flash column chromatography using ethyl acetate-hexane (3:17) as the eluent to give the *diacetate* **48** as an orange/brown oil (33.11 g, 59%); R_f 0.69 (EtOAc:hexane, 1:1); ν_{max}(neat)/cm^{-1} 3075 (C=C-H), 2992 and 2951 (CH), 1737 (C=O), 1234 (CO); δ_H(250 MHz; $CDCl_3$) 6.07 (2H, d, *J* 1.0, 2-H and 3-H), 5.52 (2H, ddd, *J* 1.0, 4.0 and 7.5, 1-H and 4-H), 2.85 (1H, dt, *J* 7.5 and 15.0, C-5-H_{2endo}), 2.03 (6H, s, 2 x CH_3), 1.71 (1H, dt, *J* 4.0 and 15.0, C-5-H_{2exo}); δ_C(63 MHz; $CDCl_3$) 170.6 (2 x C=O), 134.4 (2 x =CH), 76.4 (2 x CHO), 36.9 (CH_2), 21.0 (2 x CH_3); *m/z* (FAB) 185 (MH^+, 9%), 141 (M-CH_3CO, 2), 125 (M-$CH_3C(O)O$, 100), 43 (CH_3CO, 48); [Found: MH^+, 185.08163. $C_9H_{12}O_4$ requires MH, 185.08138]. Spectroscopic data was consistent with that stated in the literature.[93]

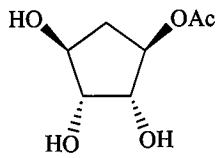
(c) (1*S*,4*R*)-4-Acetoxy-1-hydroxycyclopent-2-ene 49[52]



The diacetate **48** (9.94 g, 54.0 mmol, 1.0 eq) was added to a suspension of Novozyme® SP435 (2.0 g) in 50 mM phosphate buffer (pH = 8) (600 ml) and the resulting yellow mixture was stirred at room temperature for 4 h. The mixture was filtered to remove the catalyst, which was rinsed with ethyl acetate (50 ml). The filtrate was then saturated with sodium chloride and extracted with ethyl acetate (3 x 500 ml). The organic portions were dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (1:1) as the eluent to give the *monoacetate* **49** as an off-white solid (4.97 g, 85% based on recovered starting material); mp 45-48°C (lit.[96] mp 46-48.5°C); (Found: C, 58.86; H, 6.94. $C_7H_{10}O_3$ requires C, 59.14; H, 7.09%); R_f 0.42 (EtOAc:hexane, 1:1); Optical purity >99% e.e. (determined by chiral GC); $[\alpha]_D^{24}$ +67.4 (*c* 1.12 in $CHCl_3$); ν_{max}(nujol)/cm^{-1} 3328 (OH), 1726 (C=O), 1243 (CO); δ_H(200 MHz; $CDCl_3$) 6.09 (1H, ddd, *J* 1.0, 2.0 and 5.5, 3-H), 5.96 (1H, ddd, *J* 1.0, 2.0 and 5.5, 2-H), 5.48 (1H, m, 4-H), 4.69 (1H, m, 1-H), 2.78 (1H, dt, *J* 7.5 and 14.5, C-5-H_{2endo}), 2.03 (3H, s, CH_3), 1.92 (1H, br s, OH), 1.63 (1H, dt, *J* 4.0 and 14.5, C-5-H_{2exo}); δ_C(50 MHz; $CDCl_3$) 170.7 (C=O), 138.4

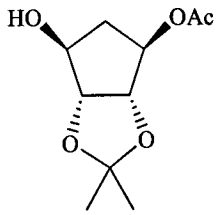
(=CH), 132.4 (=CH), 76.9 (CHO), 74.7 (CHO), 40.4 (CH_2), 21.1 (CH_3); *m/z* (FAB) 143 (MH^+, 37%), 125 (M-OH, 43), 99 (M-CH_3CO, 3), 83 (M-$CH_3C(O)O$, 37), 43 (CH_3CO, 19); [Found: MH^+, 143.07041. $C_7H_{10}O_3$ requires MH, 143.07082].

(d) (1*S*,2*R*,3*R*,4*R*)-4-Acetoxy-cyclopentan-1,2,3-triol 122



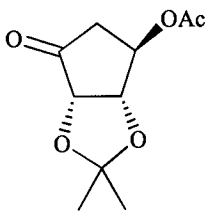
Acetone (60 ml) and water (21 ml) were added to a solution of the monoacetate **49** (15.30 g, 0.11 mol, 1.0 eq) in THF (150 ml). NMO (24.00 g, 0.18 mol, 1.6 eq) followed by osmium tetroxide (4 wt. % solution in water, 0.69 ml, 0.109 mmol, 0.1 mol %) were then added and the resulting yellow solution was stirred at room temperature for 19 h. Sodium bisulfite (1.65 g) was added to the solution, which was then concentrated under reduced pressure. The resulting brown oil was purified by flash column chromatography using methanol-ethyl acetate (1:19) as the eluent to give the *triol* **122** as a yellow oil, which formed an off-white oily solid when stored in the freezer (16.62 g, 88%); R_f 0.57 (MeOH:EtOAc, 1:9); $[\alpha]_D^{24}$ –41.4 (*c* 1.00 in MeOH) lit.[119] $[\alpha]_D^{25}$ –44.3 (*c* 1.30 in MeOH); ν_{max}(nujol)/cm^{-1} 3370 (OH), 1715 (C=O), 1263 (CO); δ_H(250 MHz; D_2O) 4.87 (1H, dt, *J* 5.0 and 8.0, 4-H), 4.14 (1H, t, *J* 5.0, 3-H), 4.06 (1H, dt, *J* 5.0 and 8.0, 1-H), 3.92 (1H, t, *J* 5.0, 2-H), 2.64 (1H, dt, *J* 8.0 and 15.0, C-5-H_{2endo}), 2.07 (3H, s, CH_3), 1.49 (1H, dt, *J* 5.0 and 15.0, C-5-H_{2exo}); δ_C(63 MHz; D_2O) 173.9 (C=O), 77.8 (CH), 76.6 (CH), 75.0 (CH), 73.8 (CH), 35.1 (CH_2), 20.5 (CH_3); *m/z* (FAB) 177 (MH^+, 100%), 159 (M-OH, 9), 117 (M-$CH_3C(O)O$, 5), 43 (CH_3CO, 13); [Found: MH^+, 177.07644. $C_7H_{12}O_5$ requires MH, 177.07630].

(e) (1*S*,2*R*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-acetoxy-cyclopentan-1-ol 117



A solution of the triol **122** (15.50 g, 88.0 mmol, 1.0 eq) and *p*-toluenesulfonic acid (300 mg, 1.58 mmol, 0.02 eq) in acetone (300 ml) was stirred at room temperature for 19 h. Calcium hydroxide was then added until a pH = 7 was obtained and the mixture was stirred at room temperature for 10 min before being filtered and the filtrate concentrated under reduced pressure to give the *acetonide-protected diol* **117** as a yellow oil (17.64 g, 93%); R_f 0.68 (MeOH:EtOAc, 1:9); $[\alpha]_D^{24}$ –12.4 (*c* 1.05 in $CHCl_3$) lit.[93] $[\alpha]_D^{25}$ –12.7 (*c* 0.95 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3464 (OH), 2988 and 2938 (CH), 1736 (C=O), 1376 (CMe_2), 1245 (CO); δ_H(250 MHz; $CDCl_3$) 5.11 (1H, d, *J* 5.0, 3-H), 4.60 (1H, dd, *J* 1.5 and 5.0, 4-H), 4.56 (1H, dd, *J* 1.5 and 5.0, 1-H), 4.20 (1H, d, *J* 5.0, 2-H), 2.31 (1H, dt, *J* 5.0 and 15.0, C-5-H_{2endo}), 2.05 (3H, s, $OC(O)CH_3$), 1.83 (1H, dt, *J* 1.5 and 15.0, C-5-H_{2exo}), 1.39 (3H, s, CCH_3), 1.25 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 169.5 (C=O), 110.6 (CMe_2), 86.1 (CH), 84.1 (CH), 79.5 (CH), 76.7 (CH), 36.1 (CH_2), 26.1 (CH_3), 23.7 (CH_3), 21.0 ($OC(O)CH_3$); *m/z* (FAB) 217 (MH^+, 67%), 201 (M-CH_3, 44), 157 (M-$CH_3C(O)O$, 35), 59 ($CH_3C(O)O$, 36), 43 (CH_3CO, 100); [Found: MH^+, 217.10782. $C_{10}H_{16}O_5$ requires MH, 217.10760].

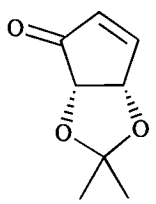
(f) (2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-acetoxy-cyclopentan-1-one 118



A solution of the acetonide-protected diol **117** (3.25 g, 15.0 mmol, 1.0 eq) in acetone (170 ml) was cooled to 0°C. Jones reagent (8.64 ml, 11.6 mmol, 0.77 eq) was added dropwise and the solution was stirred at 0°C for 15 min after which time the cooling bath was removed and the mixture was stirred at room temperature for 3

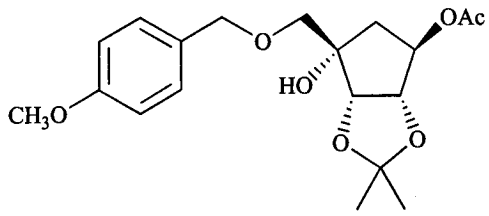
h. Sodium bicarbonate (0.4 g) and sodium bisulfite (0.4 g) were then added and the mixture was filtered and the filtrate concentrated under reduced pressure. The concentrated residue was dissolved in ethyl acetate (80 ml) and the solution was washed with brine (70 ml). The aqueous portion was further extracted with ethyl acetate (2 x 50 ml) and the combined organic portions were dried over sodium sulfate, filtered and concentrated to give a pale yellow solid. Recrystallisation of the solid from diethyl ether gave the *β-acetoxycyclopentanone* **118** as a white solid (1.92 g, 60%); mp 74-77°C; (Found: C, 55.94; H, 6.58. $C_{10}H_{14}O_5$ requires C, 56.07; H, 6.59%); R_f 0.63 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ +156.4 (*c* 1.04 in $CHCl_3$); ν_{max}(nujol)/cm^{-1} 1757 (C=O), 1730 (C=$O_{acetate}$), 1378 (CMe_2), 1257 (CO); δ_H(250 MHz; $CDCl_3$) 5.26 (1H, dt, *J* 1.5 and 6.5, 4-H), 4.67 (1H, d, *J* 5.5, 2-H), 4.36 (1H, dd, *J* 1.5 and 5.5, 3-H), 2.96 (1H, dd, *J* 6.5 and 19.0, C-5-H_{2endo}), 2.35 (1H, dd, *J* 1.5 and 19.0, C-5-H_{2exo}), 2.05 (3H, s, OC(O)CH_3), 1.42 (3H, s, CCH_3), 1.34 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 210.2 (C=O), 169.8 (C=$O_{acetate}$), 112.9 (CMe_2), 80.2 (CH), 78.0 (CH), 71.0 (CH), 40.1 (CH_2), 26.5 (CH_3), 24.5 (CH_3), 20.7 (OC(O)CH_3); *m/z* (FAB) 215 (MH^+, 42%), 155 (M-$CH_3C(O)O$, 12), 43 (CH_3CO, 74); [Found: MH^+, 215.09207. $C_{10}H_{14}O_5$ requires MH, 215.09195].

(g) (4*S*,5*S*)-4,5-(Isopropylidenedioxy)-cyclopent-2-en-1-one 61



DBU (0.5 ml, 3.34 mmol, 0.65 eq) was added to a stirred solution of the β-acetoxycyclopentanone **118** (1.10 g, 5.13 mmol, 1.0 eq) in diethyl ether (10 ml). The mixture was then stirred at room temperature for 30 min before being filtered through a pad of silica and the filtrate concentrated under reduced pressure. The resulting oily white solid was purified by flash column chromatography using ethyl acetate-hexane (1:3) as the eluent to give the *enone* **61** as a white solid (723 mg, 91%); mp 67-68°C (lit.[93] mp 68-69°C); R_f 0.57 (EtOAc:hexane, 1:1). Spectroscopic data was consistent with that for procedure 5.3.1 (d).

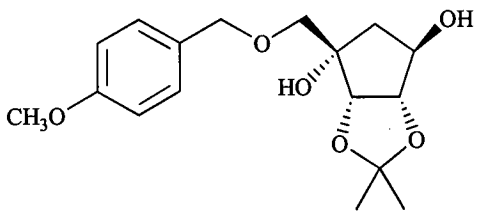
(h) (1*R*,2*R*,3*S*,4*S*)-2,3-(Isopropylidenedioxy)-4-[(*p*-methoxybenzyloxy)methyl]-1-acetoxy-cyclopentan-4-ol 120



A solution of [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** (5.48 g, 12.4 mmol, 1.05 eq) in anhydrous THF (60 ml) was cooled to -78°C. *n*-BuLi (1.61 M solution in hexanes, 7.25 ml, 11.7 mmol, 1.0 eq) was then added dropwise over 2 min to give a pale yellow solution which was stirred at -78°C for 5 min. A solution of the β-acetoxycyclopentanone **118** (2.53 g, 11.8 mmol, 1.0 eq) in anhydrous THF (38 ml) was then added *via* nitrogen pressure transfer through a double tipped needle. The resulting pale yellow solution was stirred at -78°C for 30 min after which time the reaction mixture was quenched by the addition of saturated aqueous ammonium chloride (60 ml). The mixture was allowed to warm to room temperature before being poured into ethyl acetate (100 ml). The organic layer was separated and the aqueous portion was extracted with ethyl acetate (3 x 100 ml). The combined organic portions were washed with water (250 ml) and brine (250 ml). The water portion was saturated with sodium chloride and then extracted with ethyl acetate (3 x 200 ml). The combined organic portions were dried over sodium sulfate, filtered and concentrated. The resulting colourless liquid containing a yellow oil was purified by flash column chromatography using ethyl acetate-hexane (1:3) as the eluent to give the *PMB-protected acetate* **120** as a yellow oil (822 mg, 19%); R_f 0.53 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ +2.25 (*c* 1.02 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3513 (OH), 2988, 2937 and 2861 (CH), 1739 (C=O), 1613 ($C=C_{ar}$), 1514 ($C=C_{ar}$), 1374 (CMe_2), 1246 (CO); δ_H(250 MHz; $CDCl_3$) 7.25 (2H, d, *J* 8.5, 2 x H_{ar}), 6.87 (2H, d, *J* 8.5, 2 x H_{ar}), 5.08 (1H, dd, *J* 5.0 and 6.5, 1-H), 4.52 (2H, 2d, *J* 5.5 and 5.5, 2-H and 3-H), 4.47 (2H, s, $PhCH_2$), 3.79 (3H, s, OCH_3), 3.39 (2H, s, CH_2O), 2.96 (1H, br s, OH), 2.23 (1H, dd, *J* 6.5 and 14.5, C-6-H_{2endo}), 1.95 (3H, s, $OC(O)CH_3$), 1.92 (1H, dd, *J* 5.0 and 14.5, C-6-H_{2exo}), 1.52 (3H, s, CCH_3), 1.32 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 169.7 (C=O), 159.2 (C_{ar}), 129.9 (C_{ar}), 129.3 (2 x CH_{ar}), 113.7 (2 x CH_{ar}),

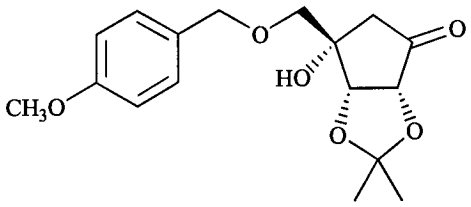
112.9 (CMe_2), 84.4 (CH), 79.9 (CH), 78.1 (C-4), 76.4 (C-1H), 73.3 (CH_2), 73.1 (CH_2), 55.1 (OCH_3), 39.3 (C-$6H_2$), 26.1 (CH_3), 24.4 (CH_3), 20.8 ($OC(O)CH_3$); *m/z* (EI) 366 (M^+, 5%), 308 (M-$CH_3C(O)O$, 4), 137 ($CH_3OC_6H_4CH_2O$, 35), 122 ($CH_3OC_6H_4CH_3$, 38), 59 ($CH_3C(O)O$, 35), 43 (CH_3CO, 100); [Found: M^+, 366.16817. $C_{19}H_{26}O_7$ requires M, 366.16785].

(i) (1*R*,2*S*,3*S*,4*S*)-2,3-(Isopropylidenedioxy)-4-[(*p*-methoxybenzyloxy)methyl]-cyclopentan-1,4-diol 123



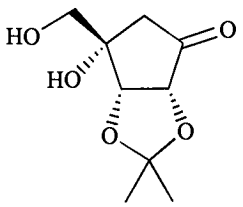
A solution of the PMB-protected acetate **120** (558 mg, 1.52 mmol) in ammonia saturated methanol (50 ml) was stirred at room temperature for 18 h 30 min after which time the solution was concentrated under reduced pressure. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (1:1) as the eluent to give *PMB-protected alcohol* **123** as a pale yellow oil (453 mg, 92%); R_f 0.38 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ +24.8 (*c* 1.20 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3445 (OH), 2987, 2936, 2864 and 2839 (CH), 1613 ($C{=}C_{ar}$), 1515 ($C{=}C_{ar}$), 1374 (CMe_2), 1248 (CO); δ_H(250 MHz; $CDCl_3$) 7.23 (2H, d, *J* 8.5, 2 x H_{ar}), 6.88 (2H, d, *J* 8.5, 2 x H_{ar}), 4.51 (2H, s, $PhCH_2$), 4.40 (2H, 2d, *J* 5.5 and 5.5, 2-H and 3-H), 4.04 (1H, m, 1-H), 3.79 (3H, s, OCH_3), 3.57 (1H, br s, C-1-OH), 3.53 (1H, d, *J* 8.5, CH_2O), 3.45 (1H, d, *J* 8.5, CH_2O), 3.14 (1H, br s, C-4-OH), 2.21 (1H, dd, *J* 6.0 and 14.5, C-6-H_{2endo}), 1.96 (1H, dd, *J* 2.0 and 14.5, C-6-H_{2exo}), 1.48 (3H, s, CCH_3), 1.32 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 159.5 (C_{ar}), 129.7 (2 x CH_{ar}), 128.5 (C_{ar}), 113.9 (2 x CH_{ar}), 111.7 (CMe_2), 87.7 (CH), 81.7 (CH), 77.9 (C-4), 75.1 (CH_2), 73.5 (CH_2), 73.1 (C-1H), 55.1 (OCH_3), 44.5 (C-$6H_2$), 26.1 (CH_3), 24.3 (CH_3); *m/z* (EI) 324 (M^+, 9%), 203 (M-$CH_3OC_6H_4CH_2$, 4), 137 ($CH_3OC_6H_4CH_2O$, 27), 121 ($CH_3OC_6H_4CH_2$, 100); [Found: M^+, 324.15759. $C_{17}H_{24}O_6$ requires M, 324.15729].

(j) (2*R*,3*S*,4*S*)-2,3-(Isopropylidenedioxy)-4-[(*p*-methoxybenzyloxy)methyl]-4-hydroxy-cyclopentan-1-one 124



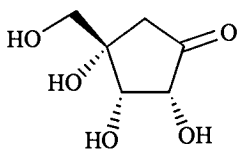
A mixture of IBX **141** (1.21 g, 4.32 mmol, 3.1 eq) in DMSO (8 ml) was stirred at room temperature for 25 min after which time the IBX had dissolved. The PMB-protected alcohol **123** (453 mg, 1.40 mmol, 1.0 eq) dissolved in the minimum volume of THF was added to the IBX solution which was then stirred at room temperature for 6 h 30 min. Water (16 ml) was added and the white solid that precipitated was filtered off. The filtrate was transferred to a separating funnel and the organic layer was separated. The aqueous portion was extracted with diethyl ether (3 x 70 ml) and the combined organic portions were dried over sodium sulfate, filtered and concentrated. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (7:13) as the eluent to give the *PMB-protected β-hydroxycyclopentanone* **124** as a pale yellow oil (397 mg, 88%); R_f 0.42 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ –89.1 (*c* 1.14 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3490 (OH), 2988, 2936 and 2865 (CH), 1759 (C=O), 1612 (C=C_{ar}), 1514 (C=C_{ar}), 1376 (CMe_2), 1248 (CO); δ_H(250 MHz; $CDCl_3$) 7.15 (2H, d, *J* 9.0, 2 x H_{ar}), 6.86 (2H, d, *J* 9.0, 2 x H_{ar}), 4.52 (1H, d, *J* 5.5, 3-H), 4.41 (2H, s, $PhCH_2$), 4.35 (1H, ddd, *J* 1.0, 1.5 and 5.5, 2-H), 3.79 (3H, s, OCH_3), 3.50 (1H, d, *J* 8.5, CH_2O), 3.42 (1H, d, *J* 8.5, CH_2O), 2.84 (1H, br s, OH), 2.69 (1H, dd, *J* 1.0 and 18.0, C-6-H_{2endo}), 2.44 (1H, ddd, *J* 1.0, 1.5 and 18.0, C-6-H_{2exo}), 1.47 (3H, s, CCH_3), 1.35 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 208.2 (C=O), 159.3 (C_{ar}), 129.3 (2 x CH_{ar}), 128.9 (C_{ar}), 113.8 (2 x CH_{ar}), 112.8 (CMe_2), 81.7 (CH), 80.8 (CH), 74.1 (CH_2), 73.3 (CH_2), 73.2 (C-4), 55.1 (OCH_3), 46.1 (C-6H_2), 26.5 (CH_3), 24.8 (CH_3); *m/z* (EI) 322 (M^+, 10%), 201 (M-$CH_3OC_6H_4CH_2$, 4), 137 ($CH_3OC_6H_4CH_2O$, 7), 121 ($CH_3OC_6H_4CH_2$, 100); [Found: M^+, 322.14213. $C_{17}H_{22}O_6$ requires M, 322.14164].

(k) (2*R*,3*S*,4*S*)-2,3-(Isopropylidenedioxy)-4-hydroxymethyl-4-hydroxy-cyclopentan-1-one 119



Palladium on activated charcoal (10% Pd, 430 mg, 0.043 g of Pd, 0.404 mmol, 0.5 eq) was added to a stirred solution of the PMB-protected β-hydroxycyclopentanone **124** (259 mg, 0.803 mmol, 1.0 eq) in anhydrous THF (8 ml). The mixture was then vigorously stirred under an atmosphere of hydrogen for 1 h 50 min. The mixture was filtered through Celite, the Celite was washed repeatedly with ethyl acetate, and the filtrate was concentrated under reduced pressure. The resulting pale yellow oil was purified by flash column chromatography using ethyl acetate-hexane (17:3) as the eluent to give the *protected β-hydroxycyclopentanone* **119** as a white solid (58 mg, 36%); mp 120-124°C; (Found: C, 53.52; H, 7.41. $C_9H_{14}O_5$ requires C, 53.46; H, 6.98%); R_f 0.41 (EtOAc); $[\alpha]_D^{23}$ –140.1 (*c* 0.90 in $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3429 (OH), 2989, 2936 and 2880 (CH), 1759 (C=O), 1376 (CMe_2), 1215 (CO); δ_H(250 MHz; $CDCl_3$) 4.67 (1H, d, *J* 6.0, 3-H), 4.40 (1H, ddd, *J* 1.0, 1.5 and 6.0, 2-H), 3.68 (1H, d, *J* 10.5, C*H*$_2$OH), 3.56 (1H, d, *J* 10.5, C*H*$_2$OH), 3.06 (1H, br s, C-4-OH), 2.74 (1H, dd, *J* 1.0 and 18.0, C-6-H$_{2endo}$), 2.47 (1H, br s, CH$_2$O*H*), 2.46 (1H, ddd, *J* 1.0, 1.5 and 18.0, C-6-H$_{2exo}$), 1.50 (3H, s, CCH_3), 1.38 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 208.7 (C=O), 113.5 (CMe_2), 80.3 (CH), 80.2 (CH), 74.0 (C-4), 67.2 (CH_2OH), 45.7 (C-6H_2), 26.4 (CH_3), 24.7 (CH_3); *m/z* (FAB) 203 (MH^+, 45%); [Found: MH^+, 203.09180. $C_9H_{14}O_5$ requires MH, 203.09195].

(l) (2*R*,3*S*,4*S*)-4-Hydroxymethyl-2,3,4-trihydroxy-cyclopentan-1-one 116



Trifluoroacetic acid (0.8 ml) was added to a solution of the protected β-hydroxycyclopentanone **119** (152 mg, 0.752 mmol, 1.0 eq) in anhydrous dichloromethane (3.8 ml). The resulting pale yellow solution was stirred at room temperature for 3 min before being concentrated under reduced pressure to give a yellow oil. The yellow oil was purified by flash column chromatography using methanol-chloroform (3:7) as the eluent to give the desired *keto-tetrol* **116** as a colourless oil (26 mg, 21%). ^{1}H, ^{13}C NMR and mass spectrometry showed that the keto-tetrol **116** was contaminated with minor traces of the eliminated enone **82a** (8.6:1), determined by 600 MHz ^{1}H NMR analysis.

(2R,3S,4S)-4-Hydroxymethyl-2,3,4-trihydroxy-cyclopentan-1-one **116**

R_f 0.36 ($MeOH:CHCl_3$, 3:7); δ_H(600 MHz; D_2O) 4.52 (1H, dd, *J* 1.5 and 5.0, 2-H), 4.25 (1H, d, *J* 5.0, 3-H), 3.70 (2H, s, C*H*$_2$OH), 2.68 (1H, ddd, *J* 1.0, 1.5 and 19.5, C-6-H$_{2exo}$), 2.32 (1H, d, *J* 19.5, C-6-H$_{2endo}$); δ_C(91 MHz; D_2O) 218.2 (C=O), 76.9 (CH), 75.1 (C-4), 72.7 (CH), 66.0 (CH_2OH), 43.2 (C-6H_2); *m/z* (FAB) 163 (MH^+, 1%), 131 (M-CH_2OH, 100).

(2R,3R)-4-Hydroxymethyl-2,3-dihydroxy-cyclopent-4-en-1-one **82a**

R_f 0.46 ($MeOH:CHCl_3$, 3:7); δ_H(600 MHz; D_2O) 6.32 (1H, t, *J* 1.5, =CH), 4.79 (1H, obscured by D_2O residual peak, 2-H), 4.68 (1H, dd, *J* 1.5 and 19.0, C*H*$_2$OH), 4.54 (1H, dd, *J* 1.5 and 19.0, C*H*$_2$OH), 4.33 (1H, d, *J* 6.0, 3-H); δ_C(91 MHz; D_2O) 209.5 (C=O), 179.6 (C-4), 126.5 (=CH), 72.5 (CH), 69.0 (CH), 60.5 (CH_2OH); *m/z* (FAB) 145 (MH^+, 2%), 113 (M-CH_2OH, 7).

5.2.4 Feeding studies

(a) General techniques

(i) Strain used

Streptomyces citricolor mutant: CC940

(ii) Preparation of liquid medium

Arkosoy 50	60 gl^{-1}
MOPS buffer	21 gl^{-1}

Made up in tap water to 800 ml and pH adjusted to 7.0 with 10 M NaOH.

After autoclaving the following ingredients were added:

Glucose	60 gl^{-1}
Uracil	25 mgl^{-1}

(iii) HPLC conditions for detecting aristeromycin and neplanocin A

HPLC analysis was carried out using a Waters 486 Tuneable Absorbance Detector and a Waters 600E Pump and Controller. Waters Millennium Chromatography Manager software package was used to analyse the results.

Column:	Phenomenex sphereclone 5 μ ODS (2) C18 reverse phase 250 x 4.6 mm	
Mobile Phase:	97% 50 mM ammonium formate buffer (pH = 3.0) 3% acetonitrile	
Flow rate:	1ml min^{-1}	
Injection:	*via* 50 μl injection loop	
Detector:	UV at 260 nm	
Retention times:	Neplanocin A	6.51 min
	Aristeromycin	9.72 min

(b) General procedure for running a feeding experiment

All bacteriological procedures were carried out in a SterilGard Class II Type A/B3 biological safety cabinet (Baker Company). The GAM 6:6 medium (without glucose) was sterilised by autoclaving at 121°C for 20 min. In order to prevent caramelisation, glucose was subsequently added to the cooled medium from a filter sterilised stock solution (50% w/v).

Streptomyces citricolor CC940 mutant was maintained as a vegetative mycelium in 20% (v/v) glycerol at -20°C. A 50 µl aliquot of mycelium was used to inoculate a 250 ml conical flask containing 60 ml of GAM 6:6 medium. The flask was fitted with a stainless steel spring to ensure the culture was sufficiently aerated and well dispersed. Following 3 days of growth at 30°C, 270 rpm, 5 ml aliquots of culture broth was transferred to two sterile 100 ml conical flasks fitted with steel coils. The substrate (20 mg) was dissolved in distilled water (1 ml) and using a sterile pipette, 250 µl of this substrate solution was transferred into one of the conical flasks. The second flask was used as a control. The flasks were then incubated at 30°C, 270 rpm and the transformation was monitored by HPLC.

At regular intervals, using sterile techniques, 430 µl was removed from each flask and placed in an eppendorf tube. This was then centrifuged at 14,000 rpm for 5 min. The supernatant was removed using a 1 ml disposable plastic syringe and the sample was filtered through a 0.2 µM syringe filter before being injected into the HPLC under the conditions outlined above.

The above procedure was used to carry out the following feeding experiments:

Feeding Experiment 1

Substrate used: Tetrol **83a** 5 mg in 5 ml of media.

Four samples removed. Final sample removed after incubation for 47 h.

Conversion after 47 h:	Neplanocin A	4.3%
	Aristeromycin	1.2%
	Total conversion	5.5%

The fraction corresponding to neplanocin A was collected and subjected to mass spectrometry: *m/z* (ES+) 263.8 (MH^+, 30%), 285.9 (M+Na, 50).

Feeding Experiment 2

Substrates used:	Tetrol **83a**	5 mg in 5 ml of media
	2*R*,3*S*,4*R* Keto-tetrol **95**	5 mg in 5 ml of media
	2*S*,3*R*,4*S* Keto-tetrol ***ent*-95**	5 mg in 5 ml of media

Four samples removed. Final sample removed after incubation for 47 h.

Tetrol **83a**:

Conversion after 47 h:	Neplanocin A	22.6%
	Aristeromycin	9.4%
	Total conversion	32.0%

2*R*,3*S*,4*R* Keto-tetrol **95**:

Conversion after 47 h:	Neplanocin A	23.7%
	Aristeromycin	9.0%
	Total conversion	32.7%

The fraction corresponding to neplanocin A was collected and subjected to mass spectrometry: *m/z* (ES+) 263.6 (MH^+, 60%), 285.7 (M+Na, 10).
The fraction corresponding to aristeromycin was collected and subjected to mass spectrometry: *m/z* (ES+) 265.8 (MH^+, 50%), 287.8 (M+Na, 20).

2*S*,3*R*,4*S* Keto-tetrol ***ent*-95**:
Shows no conversion to neplanocin A or aristeromycin.

Feeding Experiment 3

Substrates used:	Tetrol **83a**	5 mg in 5 ml of media
	2*R*,3*S*,4*R* Keto-tetrol **95**	5 mg in 5ml of media
	2*R*,3*S*,4*S* Keto-tetrol **116**	5 mg in 5 ml of media

Seven samples removed. Final sample removed after incubation for 71 h.

Tetrol **83a**:

Conversion after 71 h:	Neplanocin A	24.6%
	Aristeromycin	10.0%
	Total conversion	34.6%

2*R*,3*S*,4*R* Keto-tetrol **95**:

Conversion after 71 h:	Neplanocin A	26.7%
	Aristeromycin	10.2%
	Total conversion	36.9%

2*R*,3*S*,4*S* Keto-tetrol **116**:

Conversion after 71 h:	Neplanocin A	13.4%
	Aristeromycin	7.7%
	Total conversion	21.1%

Feeding Experiment 4

Substrates used:	Tetrol **83a**	15 mg in 15 ml of media
	2*R*,3*S*,4*R* Keto-tetrol **95**	15 mg in 15ml of media
	2*R*,3*S*,4*S* Keto-tetrol **116**	15 mg in 15 ml of media

Fifteen samples removed. Final sample removed after incubation for 143 h.

Tetrol **83a**:

Conversion after 143 h:	Neplanocin A	29.7%
	Aristeromycin	26.7%
	Total conversion	56.4%

2*R*,3*S*,4*R* Keto-tetrol **95**:

Conversion after 143 h:	Neplanocin A	8.0%
	Aristeromycin	11.6%
	Total conversion	19.6%

2*R*,3*S*,4*S* Keto-tetrol **116**:

Conversion after 143 h:	Neplanocin A	8.2%
	Aristeromycin	11.1%
	Total conversion	19.3%

Feeding Experiment 5

Substrates used:	Tetrol **83a**	5 mg in 5 ml of media
	Enone **82a**	5 mg in 5ml of media
	2*R*,3*S*,4*R* Keto-tetrol **95**	5 mg in 5 ml of media

Five samples removed. Final sample removed after incubation for 89 h.

Tetrol **83a**:

Conversion after 89 h:	Neplanocin A	25.7%
	Aristeromycin	11.5%
	Total conversion	37.2%

Enone **82a**:

Conversion after 89 h:	Neplanocin A	22.6%
	Aristeromycin	6.0%
	Total conversion	28.6%

2*R*,3*S*,4*R* Keto-tetrol **95**:

Conversion after 89 h:	Neplanocin A	15.2%
	Aristeromycin	7.0%
	Total conversion	37.2%

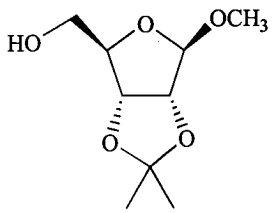
After incubation for 93 h 30 min a further 5 mg of the 2*R*,3*S*,4*R* keto-tetrol **95** was added to the media and after incubating for a further 43 h 30 min a further sample was taken.

Conversion after 137 h:	Neplanocin A	25.0%
	Aristeromycin	12.6%
	Total conversion	37.6%

5.3 Experimental Procedures for Chapter 3

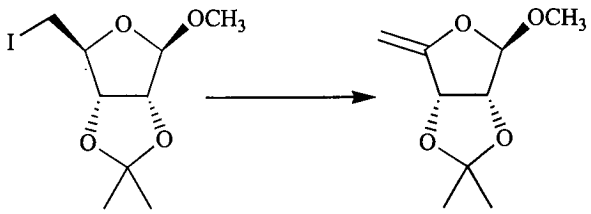
5.3.1 Synthesis of tetrol 83a *via* methoxymethyl-protected compounds

(a) Methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside 131[63]



A solution of D-ribose **126** (52.11 g, 0.35 mol, 1.0 eq) and 2,2-dimethoxypropane (100 ml, 0.81 mol, 2.3 eq) in acetone (400 ml) was cooled to 0°C and perchloric acid (70%, 4 ml) was added dropwise. On complete addition the cooling bath was removed and the contents were stirred at room temperature for 14 h. Methanol (70 ml) was added and the reaction mixture was stirred at room temperature for a further 2 h, before being cooled to 0°C and neutralised with cold saturated aqueous sodium carbonate (90 ml) at which point a white solid precipitated. The solid was removed by filtering through Celite and the filtrate was concentrated under reduced pressure. The concentrated residue was dissolved in diethyl ether (300 ml) and washed with water (2 x 100 ml) then brine (100 ml). The aqueous layer was saturated with sodium chloride and extracted with diethyl ether (5 x 200 ml). The combined organic layers were dried over sodium sulfate, filtered and concentrated to give a yellow oil. Distillation at 0.03 mmHg (60°C) gave the *protected ribose* **131** as a pale yellow oil (57.20 g, 81%); R_f 0.40 (EtOAc:PE, 1:1); $[\alpha]_D^{24}$ –78.6 (*c* 1.50 in $CHCl_3$) lit.[120] $[\alpha]_D^{25}$ –82.2 (*c* 2.00 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3458 (OH), 2988 and 2918 (CH), 1380 (CMe_2), 1241 (CO); δ_H(200 MHz; $CDCl_3$) 4.96 (1H, s, 1-H), 4.82 (1H, d, *J* 6.0, 2-H), 4.57 (1H, d, *J* 6.0, 3-H), 4.41 (1H, t, *J* 3.0, 4-H), 3.64 (2H, m, CH_2), 3.42 (3H, s, OCH_3), 3.26 (1H, br s, OH), 1.47 (3H, s, CCH_3), 1.30 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 111.9 (CMe_2), 109.7 (CH), 88.0 (CH), 85.5 (CH), 81.3 (CH), 63.7 (CH_2), 55.2 (OCH_3), 26.1 (CH_3), 24.5 (CH_3); *m/z* (FAB) 205 (MH^+, 40%), 173 (M-CH_2OH, 100); [Found: MH^+, 205.10785. $C_9H_{16}O_5$ requires MH, 205.10760].

(b) Methyl 5-deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranoside 133[97]



Method 1: Preparation of iodide using iodine, imidazole and triphenylphosphine.

Iodine (77.69 g, 0.31 mol, 1.2 eq) was added in small portions to a stirred solution of the protected ribose **131** (51.45 g, 0.25 mol, 1.0 eq), imidazole (21.99 g, 0.32 mol, 1.3 eq) and triphenylphosphine (79.38 g, 0.30 mol, 1.2 eq) in diethyl ether:acetonitrile (3:1) (930 ml). The mixture was stirred for 15 h before being filtered through Celite. The filtrate was then concentrated under reduced pressure to give the *crude iodide* **132**.

The crude iodide **132** was dissolved in anhydrous THF (600 ml) and DBU (60 ml) was added. The mixture was heated at reflux under nitrogen for 16 h before being allowed to cool to room temperature. The reaction mixture was filtered through Celite and the filtrate was concentrated. The residue was dissolved in diethyl ether (300 ml) and washed with water (2 x 250 ml) and saturated aqueous sodium thiosulfate (150 ml). The organic portion was then dried over magnesium sulfate, filtered and concentrated to give a yellow oil. Distillation at 0.03 mmHg (28-30°C) gave the *enol ether* **133** as a colourless oil (29.82 g, 64% from protected ribose **131**); R_f 0.58 (EtOAc:PE, 1:3); $[\alpha]_D^{24}$ +62.1 (*c* 1.00 in $CHCl_3$) lit.[97] $[\alpha]_D^{27}$ +60.2 (*c* 1.22 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 2989 and 2918 (CH), 1672 (C=C), 1383 (CMe_2), 1258 (CO); δ_H(200 MHz; $CDCl_3$) 5.13 (1H, s, 1-H), 5.04 (1H, d, *J* 6.0, =C*H*H), 4.62 (1H, m, 2-H), 4.52 (1H, d, *J* 6.0, =CH*H*), 4.40 (1H, m, 3-H), 3.43 (3H, s, OCH_3), 1.49 (3H, s, CCH_3), 1.37 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 161.0 (=C), 112.9 (CMe_2), 108.1 (CH), 88.4 (CH_2), 82.4 (CH), 78.5 (CH), 55.4 (OCH_3), 26.5 (CH_3), 25.4 (CH_3); *m/z* (FAB) 187 (MH^+, 23%); [Found: MH^+, 187.09714. $C_9H_{14}O_4$ requires MH, 187.09703].

Method 2: Preparation of iodide *via* tosylate.

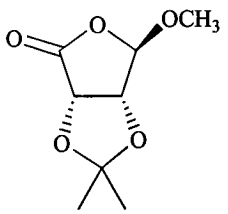
p-Toluenesulfonyl chloride (16.88 g, 88.5 mmol, 2.5 eq) and anhydrous pyridine (7.80 ml, 96.4 mmol, 2.7 eq) were added to a solution of the protected ribose **131** (7.17 g, 35.1 mmol, 1.0 eq) in chloroform (77 ml) at 0°C. The cooling bath was removed and the reaction mixture was stirred at room temperature for 30 min after which time DMAP (50 mg, cat.) was added. The resulting pale yellow solution was stirred at room temperature for 18 h before further DMAP (50 mg, cat.), *p*-toluenesulfonyl chloride (6.69 g, 35.1 mmol, 1.0 eq) and anhydrous pyridine (2.80 ml, 34.6 mmol, 1.0 eq) were added. Stirring was continued for 3 h and then water (50 ml) was added and the mixture was stirred for 15 min. The reaction mixture was poured into diethyl ether (200 ml) and washed with water (2 x 125 ml). The aqueous portions were saturated with sodium chloride and extracted with diethyl ether (150 ml). The combined organic layers were dried over magnesium sulfate, filtered and concentrated. Excess pyridine was then removed by azeotropic distillation with toluene (2 x 50 ml) to give the *crude tosylate* **139** as a yellow oil (22.97 g).

A solution of the crude tosylate **139** (22.97 g, 64.1 mmol, 1.0 eq) and sodium iodide (31.67 g, 0.21 mol, 3.3 eq) in acetone (250 ml) was heated at reflux for 16 h. TLC examination after this time showed that starting material remained, therefore further sodium iodide (30.42 g, 0.20 mol, 3.2 eq) was added and the mixture was heated at reflux for 2 days. The mixture was then cooled to room temperature before being concentrated under vacuum. Diethyl ether (100 ml) was added to the residue, which was then filtered through Celite, and the filtrate concentrated. The concentrated residue was dissolved in ethyl acetate (500 ml) and washed with saturated aqueous sodium thiosulfate (200 ml) and water (200 ml). The organic portion was dried over sodium sulfate, filtered and concentrated to give the *crude iodide* **132** as a yellow oil (16.14 g).

The crude iodide **132** (16.14 g, 51.4 mmol, 1.0 eq) was dissolved in anhydrous THF (200 ml) and DBU (17.00 ml, 0.11 mol, 2.2 eq) was added. The mixture was heated at reflux under nitrogen for 19 h before being allowed to cool to room temperature. The reaction mixture was filtered through Celite and the filtrate was

concentrated. The residue was dissolved in diethyl ether (250 ml) and washed with water (3 x 200 ml). The aqueous portions were then further extracted with diethyl ether (2 x 200 ml). The combined organic portions were dried over magnesium sulfate, filtered and concentrated to give a yellow oil. Distillation at 0.08 mmHg (32°C) gave the *enol ether* **133** as a colourless oil (2.93 g, 45% from protected ribose **131**). Spectroscopic data was consistent with that synthesised in Method 1.

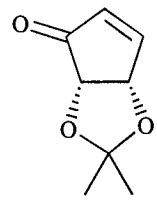
(c) (2*S*,3*R*,4*R*)-2,3-(Isopropylidenedioxy)-4-methoxybutyrolactone 134[97]



Sodium periodate (72.62 g, 0.34 mol, 2.1 eq) was added in small portions over a period of 7 h to a stirred solution of enol ether **133** (29.79 g, 0.16 mol, 1.0 eq) and osmium tetroxide (4 wt. % solution in water, 2.0 ml, cat.) in a mixture of THF (490 ml) and water (122 ml) at 0°C. The mixture was stirred at 0°C for 15 h after which time a further portion of sodium periodate (17.11 g, 80.0 mmol, 0.5 eq) was added and the mixture was allowed to warm to room temperature. After stirring at room temperature for 23 h an additional portion of sodium periodate (17.11 g, 80.0 mmol, 0.5 eq) was added and the mixture was stirred at room temperature for a further 4 h. The mixture was then filtered through Celite and the filtrate was concentrated. The residue was poured into ethyl acetate (500 ml), which was washed with brine (3 x 250 ml), water (250 ml) and saturated aqueous sodium metabisulfite (200 ml). The aqueous brine and water portions were extracted with ethyl acetate (2 x 200 ml) then the organic layers were washed with saturated aqueous sodium metabisulfite (200 ml). The combined organic layers were dried over sodium sulfate, filtered and concentrated to give an off-white solid. Recrystallisation of the solid from petroleum ether-diethyl ether gave the *lactone* **134** as white needles (20.11 g, 67%); mp 72-74°C (lit.[63] mp 75-76°C); (Found: C, 50.80; H, 6.34. $C_8H_{12}O_5$ requires C, 51.06; H, 6.43%); R_f 0.37 (EtOAc:PE, 1:3); $[\alpha]_D^{24}$ –58.3 (*c* 1.09 in MeOH); ν_{max} (nujol)/cm^{-1} 2947 and 2919 (CH), 1782 (C=O), 1382 (CMe_2), 1255 (CO); δ_H(200

MHz; $CDCl_3$) 5.27 (1H, s, 4-H), 4.74 (1H, d, *J* 5.5, 2-H), 4.48 (1H, d, *J* 5.5, 3-H), 3.47 (3H, s, OCH_3), 1.39 (3H, s, CCH_3), 1.32 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 173.5 (C=O), 114.3 (CMe_2), 104.9 (CH), 79.1 (CH), 74.3 (CH), 56.9 (OCH_3), 26.5 (CH_3), 25.5 (CH_3); *m/z* (FAB) 189 (MH^+, 43%); [Found: MH^+, 189.07579. $C_8H_{12}O_5$ requires MH, 189.07630].

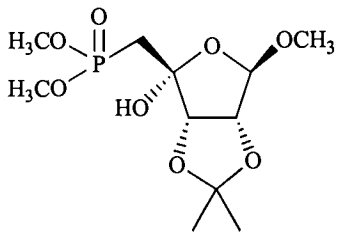
(d) (4*S*,5*S*)-4,5-(Isopropylidenedioxy)-cyclopent-2-en-1-one 61



A solution of dimethyl methylphosphonate (3.50 ml, 32.3 mmol, 1.0 eq) in anhydrous THF (150 ml) was cooled to -78°C. *n*-BuLi (1.57 M solution in hexanes, 20.30 ml, 31.9 mmol, 1.0 eq) was then added dropwise to the solution over 12 min so that the temperature did not rise above -78°C. The solution was stirred at -78°C under nitrogen for 30 min, after which time a solution of the lactone **134** (6.02 g, 32.0 mmol, 1.0 eq) in anhydrous THF (20 ml) was added *via* nitrogen pressure transfer through a double tipped needle. The solution was stirred at -78°C for a further 2 h 30 min after which time the acetone/dry ice bath was replaced by an ice/brine bath and the solution was allowed to warm up to approximately -10°C. The reaction mixture was poured into ice-cold brine (150 ml) and diethyl ether (250 ml) was added. After removal of the organic phase, the aqueous layer was extracted with diethyl ether (2 x 100 ml). The combined organic layers were washed with brine (200 ml), dried over sodium sulfate, filtered and concentrated to give the *enone* **61** as a yellow oil which formed an off-white crystalline solid when concentrated under vacuum (2.41 g, 49%); mp 66-68°C (lit.[63] mp 66-67°C); (Found: C, 62.57; H, 6.60. $C_8H_{10}O_3$ requires C, 62.33; H, 6.54%); R_f 0.54 (EtOAc:PE, 1:1); $[\alpha]_D^{24}$ +65.4 (*c* 1.02 in $CHCl_3$); ν_{max}(nujol)/cm^{-1} 2979 and 2918 (CH), 1756 (C=O), 1584 (C=C), 1377 (CMe_2), 1240 (CO); δ_H(200 MHz; $CDCl_3$) 7.60 (1H, dd, *J* 2.0 and 6.0, 3-H), 6.22 (1H, d, *J* 6.0, 2-H), 5.27 (1H, dd, *J* 2.0 and 5.5, 4-H), 4.47 (1H, d, *J* 5.5, 5-H), 1.41 (6H, s, CH_3CCH_3); δ_C(63 MHz; $CDCl_3$) 202.9 (C=O), 159.5 (=CH), 134.2

(=CH), 115.4 (CMe_2), 78.4 (CH), 76.4 (CH), 27.2 (CH_3), 26.0 (CH_3); *m/z* (FAB) 155 (MH^+, 18%); [Found: MH^+, 155.07058. $C_8H_{10}O_3$ requires MH, 155.07082].

(e) Methyl 5-deoxy-2,3-*O*-isopropylidene-4-dimethyl methylphosphonate-4-hydroxy-β-D-ribofuranoside 140



A solution of dimethyl methylphosphonate (2.30 ml, 21.2 mmol, 1.0 eq) in anhydrous THF (100 ml) was cooled to -78°C. *n*-BuLi (1.53 M solution in hexanes, 13.90 ml, 21.2 mmol, 1.0 eq) was then added dropwise to the solution over 15 min so that the temperature did not rise above -78°C. The solution was stirred at -78°C under nitrogen for 30 min, after which time a solution of the lactone **134** (3.95 g, 21.0 mmol, 1.0 eq) in anhydrous THF (10 ml) was added dropwise *via* nitrogen pressure transfer through a double tipped needle. The solution was stirred at -78°C for 2 h 30 min before being quenched with saturated aqueous ammonium chloride (35 ml). The reaction mixture was allowed to warm to room temperature before being poured into diethyl ether (200 ml). The organic layer was separated and the aqueous portion was extracted with diethyl ether (100 ml). The combined organic layers were washed with brine (2 x 150 ml), dried over sodium sulfate, filtered and concentrated to give a yellow solid. Recrystallisation of the solid from petroleum ether-diethyl ether gave the *phosphonate* **140** as a white solid (4.00 g, 61%); mp 64-65°C; (Found: C, 42.63; H, 6.84. $C_{11}H_{21}O_8P$ requires C, 42.31; H, 6.78%); R_f 0.20 (EtOAc:PE, 1:1); $[\alpha]_D^{24}$ –43.0 (*c* 1.05 in $CHCl_3$); ν_{max}(nujol)/cm^{-1} 3228 (OH), 2988 (CH), 1383 (CMe_2), 1300 (P-C), 1231 (P=O), 1116 (CO), 1042 (P-O-alkyl); δ_H(200 MHz; $CDCl_3$) 5.63 (1H, s, OH), 5.00 (1H, s, 1-H), 4.74 (1H, d, *J* 6.0, 3-H), 4.56 (1H, d, *J* 6.0, 2-H), 3.85 (3H, d, J_{PH} 11.0, $POCH_3$), 3.74 (3H, d, J_{PH} 11.0, $POCH_3$), 3.40 (3H, s, OCH_3), 2.52-2.23 (1H, q, J_{HH} 15.5 and J_{PH} 25.0, CH_2), 2.43-2.15 (1H, q, J_{HH} 15.5 and J_{PH} 25.0, CH_2), 1.44 (3H, s, CCH_3), 1.30 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$)

112.6 (CMe_2), 109.6 (C-1H), 106.4 (1C, d, J_{PC} 7.0, C-4), 85.1 (1C, d, J_{PC} 10.0, C-3H), 85.0 (C-2H), 54.9 (OCH_3), 53.2 (1C, d, J_{PC} 6.0, $POCH_3$), 51.8 (1C, d, J_{PC} 6.5, $POCH_3$), 31.1 (1C, d, J_{PC} 137.0, PCH_2), 26.1 (CH_3), 24.7 (CH_3); δ_P(101 MHz; $CDCl_3$) 31.76; *m/z* (ES) 313 (MH^+, 80%); [Found: (EI) M^+, 312.09769. $C_{11}H_{21}O_8P$ requires M, 312.09741].

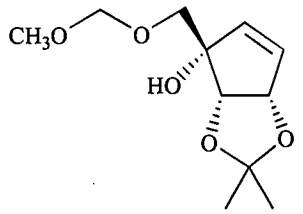
(f) [(Methoxymethoxy)methyl]tri-*n*-butylstannane **110**[102]

$$[CH_3(CH_2)_3]_3SnCH_2OCH_2OCH_3$$

To a solution of diisopropylamine (10.60 ml, 75.6 mmol, 1.1 eq) in anhydrous THF (120 ml) at 0°C was added dropwise *n*-BuLi (1.51 M solution in hexanes, 45.40 ml, 68.6 mmol, 1.0 eq). The resulting solution was stirred for 5 min at 0°C, after which time tri-*n*-butyltin hydride (18.50 ml, 68.6 mmol, 1.0 eq) was added dropwise. After stirring for 5 min at 0°C the mixture was allowed to warm to room temperature over 10 min before solid paraformaldehyde (2.06 g, 68.6 mmol, 1.0 eq) was added and the mixture was stirred at room temperature for 3 h 30 min. The pale yellow reaction mixture was poured into petroleum ether (600 ml) and washed with water (3 x 200 ml). The organic phase was dried over magnesium sulfate, filtered and concentrated to give the crude (tri-*n*-butylstannyl)methanol as a colourless liquid. This was redissolved in dichloromethane (200 ml) and dimethylaniline (18 ml) was added. The mixture was cooled to 0°C then chloromethyl methyl ether (8 ml) was added and the mixture was stirred at 0°C for 30 min, after which the reaction mixture was stirred at room temperature for 16 h. The resulting brown solution was poured into petroleum ether (600 ml) and washed with cold 0.5 M HCl (2 x 200 ml), water (2 x 200 ml) and saturated aqueous sodium bicarbonate (200 ml). The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting yellow liquid was purified by flash column chromatography using petroleum ether (500 ml) then ethyl acetate-petroleum ether (1:24) (500 ml) then ethyl acetate-petroleum ether (1:9) (500 ml) as the eluent to give the *stannane* **110** as a colourless liquid (which was stored in the fridge under an atmosphere of nitrogen) (21.39 g, 85%); R_f 0.76 (EtOAc:PE, 1:3); ν_{max}(neat)/cm^{-1} 2954, 2924, 2871 and 2859

(CH), 1145 (CO), δ_H(250 MHz; $CDCl_3$) 4.50 (2H, s, OCH_2O), 3.72 (2H, s, $SnCH_2$), 3.31 (3H, s, OCH_3), 1.59-0.81 (27H, complex m, Bu_3); δ_C(63 MHz; $CDCl_3$) 99.4 (OCH_2O), 57.5 ($SnCH_2$), 54.8 (OCH_3), 29.0 (3 x CH_2 of Bu_3), 27.2 (3 x CH_2 of Bu_3), 13.6 (3 x CH_3 of Bu_3), 8.8 (3 x CH_2 of Bu_3); *m/z* (FAB) 369 (0.3%), 368 (0.3), 367 (0.1), 366 (0.1), 365 (2.2), 364 (0.4), 363 (1.6), 362 (0.2), 361 (0.8), 313 (17), 311 (15), 310 (12), 309 (100), 308 (35), 307 (76), 306 (29), 305 (45), 45 (CH_3OCH_2, 22). Spectroscopic data was consistent with that stated in the literature.[102]

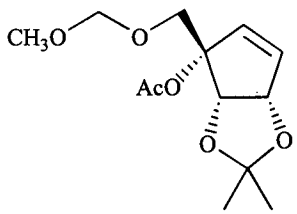
(g) (1*S*,4*S*,5*S*)-4,5-(Isopropylidenedioxy)-1-[(methoxymethoxy)methyl]-cyclopent-2-en-1-ol 135[101]



To a solution of [(methoxymethoxy)methyl]tri-*n*-butylstannane **110** (928 mg, 2.54 mmol, 1.4 eq) in anhydrous THF (7 ml) at -78°C was added *n*-BuLi (1.76 M solution in hexanes, 1.40 ml, 2.46 mmol, 1.3 eq) dropwise over a period of 2 min so as to maintain the temperature below -70°C. The solution was stirred under nitrogen for 5 min, after which time the enone **61** (282 mg, 1.83 mmol, 1.0 eq) dissolved in anhydrous THF (2 ml) was added *via* nitrogen pressure transfer through a double tipped needle. After the mixture was stirred for 30 min at -78°C, the reaction was quenched by the addition of saturated aqueous ammonium chloride (10 ml). The mixture was extracted with ethyl acetate (100 ml) and the organic layer was washed with saturated aqueous ammonium chloride (2 x 100 ml). The aqueous layers were saturated with sodium chloride then extracted with ethyl acetate (2 x 100 ml). The combined organic layers were dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting yellow oil was purified by column chromatography using ethyl acetate-petroleum ether (3:7) as the eluent to give the *alcohol* **135** as a colourless oil (301 mg, 72%); R_f 0.41 (EtOAc:PE, 1:1); $[\alpha]_D^{22}$ +77.5 (*c* 0.40 in MeOH); ν_{max}(neat)/cm^{-1} 3504 (OH), 2987 and 2946 (CH), 1372 (CMe_2), 1213 (CO); δ_H(200 MHz; $CDCl_3$) 5.91 (1H, dd, *J* 2.0 and 6.0, 3-H),

5.76 (1H, d, *J* 6.0, 2-H), 5.05 (1H, dd, *J* 2.0 and 5.5, 4-H), 4.63 (2H, s, OCH_2O), 4.51 (1H, d, *J* 5.5, 5-H), 3.62 (1H, d, *J* 10.0, $CH_2OCH_2OCH_3$), 3.48 (1H, d, *J* 10.0, $CH_2OCH_2OCH_3$), 3.34 (3H, s, OCH_3), 3.21 (1H, s, OH), 1.44 (3H, s, CCH_3), 1.39 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 136.8 (=CH), 132.8 (=CH), 112.6 (CMe_2), 96.6 (OCH_2O), 83.7 (CH), 81.4 (C-1), 80.0 (CH), 71.6 (CH_2), 55.2 (OCH_3), 27.5 (CH_3), 26.4 (CH_3); *m/z* (FAB) 231 (MH^+, 4%), 214 (MH-OH, 4), 185 ($M-CH_2OCH_3$, 5), 155 ($M-CH_2OCH_2OCH_3$, 9), 45 (CH_3OCH_2, 100); [Found: MH^+, 231.12339. $C_{11}H_{18}O_5$ requires MH, 231.12325]. Spectroscopic data was consistent with that stated in the literature.[101]

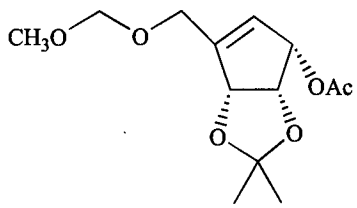
(h) (1*S*,4*S*,5*S*)-4,5-(Isopropylidenedioxy)-1-[(methoxymethoxy)methyl]-cyclopent-2-enyl-1-acetate 136[97]



DMAP (210 mg, cat.) was added to a stirred solution of alcohol **135** (1.77 g, 7.69 mmol, 1.0 eq), anhydrous pyridine (5.10 ml, 63.1 mmol, 8.2 eq) and acetic anhydride (4.10 ml, 43.5 mmol, 5.7 eq) in anhydrous dichloromethane (25 ml). After stirring the solution at room temperature under nitrogen for 2 days the reaction mixture was poured into dichloromethane (200 ml) and washed with water (3 x 100 ml). The aqueous portion was extracted with dichloromethane (2 x 150 ml) and the combined organic layers were dried over magnesium sulfate, filtered and concentrated under reduced pressure. After the removal of excess pyridine by azeotropic distillation with toluene (3 x 50 ml), the residue was purified by flash column chromatography using ethyl acetate-petroleum ether (1:4) as the eluent to give the *ester* **136** as a pale yellow oil (1.85 g, 89%); R_f 0.52 (EtOAc:PE, 1:1); $[\alpha]_D^{25}$ +114.2 (*c* 1.05 in $CHCl_3$) lit.[97] $[\alpha]_D^{23}$ +110.3 (*c* 0.22 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 2979 and 2920 (CH), 1744 (C=O), 1371 (CMe_2), 1238 (CO); δ_H(250 MHz; $CDCl_3$) 6.00 (2H, s, 2-H and 3-H), 5.03 (1H, d, *J* 5.5, 4-H), 4.76 (1H, d, *J* 5.5, 5-H), 4.55 (2H, s, OCH_2O), 3.95 (1H, d, *J* 10.0, $CH_2OCH_2OCH_3$), 3.79 (1H, d, *J* 10.0, $CH_2OCH_2OCH_3$), 3.30 (3H, s, OCH_3),

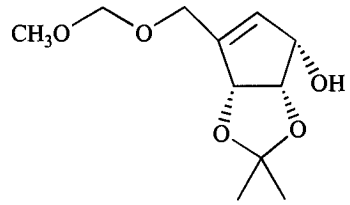
2.06 (3H, s, $OC(O)CH_3$), 1.36 (3H, s, CCH_3), 1.35 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 169.9 (C=O), 134.2 (=CH), 133.4 (=CH), 112.0 (CMe_2), 96.5 (OCH_2O), 88.5 (C-1), 83.6 (CH), 80.5 (CH), 69.1 (CH_2), 55.2 (OCH_3), 27.5 (CH_3), 26.9 (CH_3), 21.4 ($OC(O)CH_3$); *m/z* (FAB) 273 (MH^+, 22%), 45 (CH_3OCH_2, 100); [Found: MH^+, 273.13321. $C_{13}H_{20}O_6$ requires MH 273.13381].

(i) (1*S*,2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(methoxymethoxy)methyl]-cyclopent-4-enyl-1-acetate 137[97]



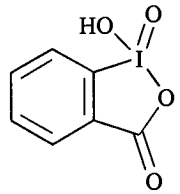
To a solution of the ester **136** (476 mg, 1.75 mmol, 1.0 eq) in anhydrous THF (15 ml) was added bis(acetonitrile)dichloropalladium(II) complex (16.2 mg, 0.062 mmol, 4 mol %) and benzoquinone (77.5 mg, 0.72 mmol, 0.4 eq). The resulting orange solution was heated at reflux under nitrogen for 6 h. After cooling to room temperature the mixture was concentrated under reduced pressure. The resulting orange/brown oil was purified by flash column chromatography using ethyl acetate-petroleum ether (1:3) as the eluent to give the *acetate* **137** as a pale yellow oil (316 mg, 66%); R_f 0.27 (EtOAc:PE, 1:3); ν_{max}(neat)/cm^{-1} 3019 (C=CH), 2937 (CH), 1734 (C=O), 1374 (CMe_2), 1216 (CO); δ_H(200 MHz; $CDCl_3$) 5.76 (1H, d, *J* 2.0, 6-H), 5.35 (1H, m, 1-H), 4.93 (2H, m, 2-H and 3-H), 4.66 (2H, s, OCH_2O), 4.26 (2H, m, $CH_2OCH_2OCH_3$), 3.37 (3H, s, OCH_3), 2.10 (3H, s, $OC(O)CH_3$), 1.38 (3H, s, CCH_3), 1.36 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 170.4 (C=O), 145.3 (C=), 126.2 (=CH), 112.8 (CMe_2), 96.0 (OCH_2O), 82.7 (CH), 77.2 (CH), 75.1 (CH), 63.4 (CH_2), 55.3 (OCH_3), 27.2 (CH_3), 26.6 (CH_3), 20.7 ($OC(O)CH_3$). ^1H and ^{13}C NMR spectra also showed minor peaks due to an impurity. The acetate was used in the next step without further purification.

(j) (1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(methoxymethoxy)methyl]-cyclopent-4-en-1-ol 130[97]



To a solution of the impure acetate **137** (817 mg, 3.00 mmol, 1.0 eq) in anhydrous methanol (28 ml) was added anhydrous potassium carbonate (2.08 g, 15.0 mmol, 5.0 eq, excess). After stirring at room temperature under nitrogen for 1 h 30 min the mixture was diluted with ethyl acetate (30 ml) and filtered through a pad of silica. The filtrate was then concentrated under reduced pressure. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-petroleum ether (1:1) as the eluent to give the *alcohol* **130** as a colourless oil (599 mg, 87%); R_f 0.41 (EtOAc:PE, 1:1). ^1H and ^{13}C NMR spectra showed the presence of the same impurity that was present in the impure acetate **137** starting material. The alcohol was not further purified but was subjected to the oxidation-reduction sequence described.

(k) *o*-Iodoxybenzoic acid 141[104]

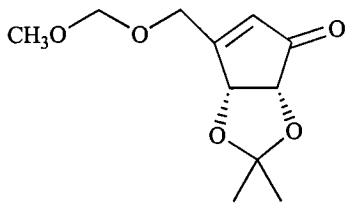


Potassium bromate (17.97 g, 0.11 mol, 1.3 eq) was added over 45 min to a mixture of 2-iodobenzoic acid (20.00 g, 81.0 mmol, 1.0 eq) in 0.73 M H_2SO_4 (500 ml) at 55°C. The mixture was stirred at 68°C for 1 h after which time the temperature was increased to 84°C and the mixture was stirred for a further 2 h 30 min. The reaction mixture was cooled in an ice bath and the solid was filtered off. The collected solid was washed with water (1 litre), acetone (500 ml) and diethyl

ether (500 ml) before being dried in a vacuum desiccater to give the *IBX* **141** as a white powder (11.60 g, 51%).

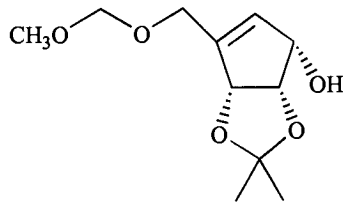
CAUTION: IBX has been reported to be explosive under heavy impact and heating over 200°C.[104]

(l) (2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(methoxymethoxy)methyl]-cyclopent-4-en-1-one 138



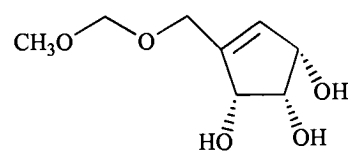
o-Iodoxybenzoic acid **141** (2.19 g, 7.82 mmol, 3.0 eq) was stirred in DMSO (16 ml) for 15 min, after which time the IBX had dissolved. The alcohol **130** (599 mg, 2.61 mmol, 1.0 eq) dissolved in the minimum amount of THF was then added to the IBX solution and the resulting pale yellow solution was stirred at room temperature for 1 h 30 min. Water (15 ml) was added to the reaction mixture and the white solid which precipitated was filtered off. The filtrate was transferred to a separating funnel and after separation of the organic portion, the aqueous layer was saturated with sodium chloride and extracted with diethyl ether (2 x 50 ml). The combined organic layers were then dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting orange oil was purified by flash column chromatography using ethyl acetate-petroleum ether (3:7) as the eluent to give the *enone* **138** as a colourless oil (517 mg, 87%); R_f 0.64 (EtOAc:PE, 1:1); $[\alpha]_D^{24}$ –13.5 (*c* 1.01 in $CHCl_3$) lit.[105] $[\alpha]_D^{20}$ –13.1 (*c* 1.10 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3019 (C=CH), 2989 and 2936 (CH), 1759 (C=O), 1376 (CMe_2), 1216 (CO); δ_H(200 MHz; $CDCl_3$) 6.15 (1H, t, *J* 2.0, 6-H), 5.10 (1H, d, *J* 5.5, 3-H), 4.70 (2H, s, OCH_2O), 4.53 (1H, dd, *J* 2.0 and 17.5, CH_2), 4.48 (1H, d, *J* 5.5, 2-H), 4.39 (1H, dd, *J* 2.0 and 17.5, CH_2), 3.39 (3H, s, OCH_3), 1.39 (6H, s, CH_3CCH_3); δ_C(63 MHz; $CDCl_3$) 201.5 (C=O), 173.4 (C=), 128.2 (=CH), 115.4 (CMe_2), 96.3 (OCH_2O), 77.9 (CH), 77.6 (CH), 64.6 (CH_2), 55.5 (OCH_3), 27.3 (CH_3), 26.1 (CH_3); *m/z* (FAB) 229 (MH^+, 10%), 45 (CH_3OCH_2, 100); [Found: MH^+, 229.10830. $C_{11}H_{16}O_5$ requires MH, 229.10760].

(m) (1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(methoxymethoxy)methyl]-cyclopent-4-en-1-ol 130[97]



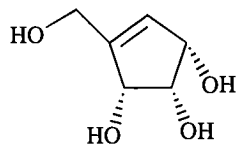
Sodium borohydride (88 mg, 2.33 mmol, 1.3 eq) was added in small portions to a stirred solution of enone **138** (419 mg, 1.84 mmol, 1.0 eq) and cerium trichloride heptahydrate (1.17 g, 3.13 mmol, 1.7 eq) in anhydrous methanol (15 ml). The mixture was stirred at room temperature for 30 min before being poured into ethyl acetate (50 ml) and washed with brine (3 x 30 ml). The aqueous portions were saturated with sodium chloride and extracted with ethyl acetate (2 x 100 ml). The combined organic layers were dried over magnesium sulfate, filtered and concentrated. The resulting pale yellow oil was purified by flash column chromatography using ethyl acetate-petroleum ether (3:7) as the eluent to give the *alcohol* **130** as a colourless oil (363 mg, 86%); R_f 0.50 (EtOAc:PE, 1:1); $[\alpha]_D^{25}$ +25.6 (*c* 0.36 in MeOH) lit.[105] $[\alpha]_D^{20}$ +36.8 (*c* 0.80 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3429 (OH), 3019 (C=CH), 2931 (CH), 1375 (CMe_2), 1215 (CO); δ_H(200 MHz; $CDCl_3$) 5.76 (1H, m, 6-H), 4.95 (1H, d, *J* 5.5, 3-H), 4.75 (1H, t, *J* 5.5, 2-H), 4.64 (2H, s, OCH_2O), 4.55 (1H, m, 1-H), 4.10-4.26 (2H, m, $CH_2OCH_2OCH_3$), 3.36 (3H, s, OCH_3), 2.70 (1H, br s, OH), 1.41 (3H, s, CCH_3), 1.39 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 142.2 (C=), 131.2 (=CH), 112.4 (CMe_2), 95.9 (OCH_2O), 82.8 (CH), 77.7 (CH), 73.2 (CH), 63.1 (CH_2), 55.2 (OCH_3), 27.5 (CH_3), 26.5 (CH_3); *m/z* (FAB) 231 (MH^+, 24%), 45 (CH_3OCH_2, 100); [Found: MH^+, 231.12341. $C_{11}H_{18}O_5$ requires MH, 231.12325].

(n) (1*S*,2*S*,3*R*)-4-[(Methoxymethoxy)methyl]-cyclopent-4-en-1,2,3-triol 142



Amberlite IR 120 (H) (2.70 g, excess) was added to a solution of alcohol **130** (55 mg, 0.24 mmol, 1.0 eq) in methanol (4 ml) and water (0.4 ml) and the mixture was stirred at room temperature for 3 h. The liquid was then decanted off and the resin was washed with methanol (3 x 5 ml). The decanted portions were combined and concentrated to give a yellow oil which was purified by flash column chromatography using methanol-chloroform (1:4) as the eluent to give the *MOM-protected triol* **142** as a colourless oil (26 mg, 57%); R_f 0.52 ($MeOH:CHCl_3$, 1:4); ν_{max}(neat)/cm^{-1} 3412 (OH), 3019 (C=CH), 2949 (CH), 1219 (CO); δ_H(250 MHz; $CDCl_3$) 5.93 (1H, d, *J* 1.5, 6-H), 4.64 (2H, s, OCH_2O), 4.43 (1H, m, 1-H), 4.37 (1H, d, *J* 5.5, 3-H), 4.22 (2H, s, $CH_2OCH_2OCH_3$), 4.08 (1H, t, *J* 5.5, 2-H), 3.36 (3H, s, OCH_3); δ_C(63 MHz; $CDCl_3$) 145.4 (C=), 130.2 (=CH), 96.0 (OCH_2O), 73.4 (CH), 73.1 (CH), 71.3 (CH), 64.1 (CH_2), 55.3 (OCH_3); *m/z* (FAB) 191 (MH^+, 26%), 159 (M-OCH_3, 48), 45 (CH_3OCH_2, 70); [Found: MH^+, 191.09261. $C_8H_{14}O_5$ requires 191.09195].

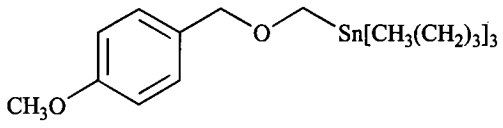
(o) (1*S*,2*S*,3*R*)-4-Hydroxymethylcyclopent-4-en-1,2,3-triol 83a



Amberlite IR 120 (H) (5.00 g, excess) was added to a solution of alcohol **130** (92 mg, 0.40 mmol, 1.0 eq) in methanol (7 ml) and water (0.7 ml) and the mixture was stirred at room temperature for 4 days. The reaction mixture was filtered through Celite; the Celite being washed with ethanol and the filtrate was concentrated under reduced pressure. The resulting yellow oil was purified by flash column chromatography using methanol-chloroform (1:3) as the eluent to give the *tetrol* **83a** as a colourless oil (30 mg, 52%); R_f 0.15 ($MeOH:CHCl_3$, 1:4); $[\alpha]_D^{24}$ +22.8 (*c* 1.10 in H_2O); ν_{max}(neat)/cm^{-1} 3378 (OH), 2949 (CH), 1676 (C=C), 1213 (CO); δ_H(250 MHz; D_2O) 5.91 (1H, m, 6-H), 4.49 (1H, m, 1-H), 4.44 (1H, d, J 5.5, 3-H), 4.32 (2H, m, CH_2), 4.16 (1H, t, J 5.5, 2-H); δ_C(63 MHz; D_2O) 147.8 (C=), 127.0 (=CH), 72.6 (CH), 72.4 (CH), 71.2 (CH), 58.1 (CH_2); *m/z* (FAB) 147 (MH^+, 10%); [Found: MH^+, 147.06557. $C_6H_{10}O_4$ requires MH, 147.06573].

5.3.2 Synthesis of tetrol 83a *via para*-methoxybenzyl-protected compounds

(a) [(*p*-Methoxybenzyloxy)methyl]tri-*n*-butylstannane 108



(*p*-Methoxybenzyl)trichloroacetimidate **147**:

A solution of *p*-methoxybenzyl alcohol (23.96 g, 0.17 mol ,1.0 eq) in anhydrous diethyl ether (30 ml) was added *via* nitrogen pressure transfer through a double tipped needle to a mixture of sodium hydride (60% dispersion in mineral oil, 0.81 g, 20.2 mmol, 12 mol %) in anhydrous diethyl ether (30 ml). The mixture was cooled to 0°C and trichloroacetonitrile (17.40 ml, 0.17 mol, 1.0 eq) was added dropwise. The resulting pale brown solution was stirred at 0°C under nitrogen for 1 h 20 min after which time the cooling bath was removed and the solution was stirred at room temperature for 2 h 20 min. The reaction mixture was concentrated then anhydrous methanol (7 ml, 0.17 mol, 1.0 eq) was added followed by pentane (70 ml). The mixture was shaken vigorously and then stirred for 10 min before being filtered through Celite. The filtrate was concentrated under reduced pressure to give the *trichloroacetimidate* **147** as a brown liquid (46.04 g).

(Tri-*n*-butylstannyl)methanol **146**:

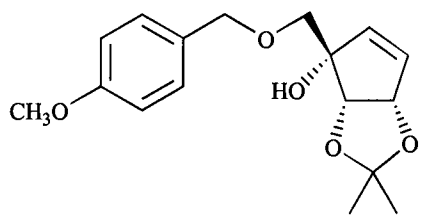
To a stirred solution of diisopropylamine (11.50 ml, 81.4 mmol, 1.1 eq) in anhydrous THF (100 ml) at 0°C was added *n*-BuLi (1.48 M solution in hexanes, 50.00 ml, 74.0 mmol, 1.0 eq) followed by tri-*n*-butyltin hydride (20.00 ml, 74.0 mmol, 1.0 eq). The cooling bath was removed and the yellow solution was allowed to warm to room temperature over 10 min before solid paraformaldehyde (2.22 g, 74.0 mmol, 1.0 eq) was added and the mixture was stirred at room temperature for 4 h. The yellow solution was poured into hexane (300 ml) and washed with water (3 x 200 ml). The organic phase was dried over sodium sulfate, filtered and

concentrated to give the *(tri-*n*-butylstannyl)methanol* **146** as a pale yellow liquid (25.56 g).

[(*p*-Methoxybenzyloxy)methyl]tri-*n*-butylstannane **108**:

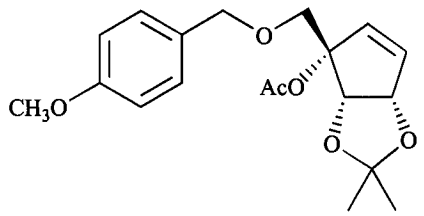
Triflic acid (1 ml, cat.) was added dropwise to a solution of (*p*-methoxybenzyl)trichloroacetimidate **147** (46.04 g, 0.16 mol, 2.04 eq) and (tri-*n*-butylstannyl)methanol **146** (25.56 g, 80.0 mmol, 1.0 eq) in anhydrous dichloromethane (100 ml). The resulting yellow solution containing white solid was stirred at room temperature under nitrogen for 2 days before being filtered through Celite. The filtrate was concentrated to give a yellow oil containing white solid to which pentane (70 ml) was added and the mixture was again filtered through Celite and the filtrate concentrated. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (1:99) (2 litres), ethyl acetate-hexane (2:98) (4 litres) then ethyl acetate-hexane (3:97) (1 litre) as the eluent to give a colourless liquid. This was further purified by distillation at 0.02 mbar (150°C) to give the *stannane* **108** as a colourless liquid (which was stored in the fridge under an atmosphere of nitrogen) (13.68 g, 42% from Bu_3SnH); R_f 0.75 (EtOAc:hexane, 1:9); ν_{max}(neat)/cm^{-1} 2956, 2925, 2871 and 2852 (CH); 1513 (C=C); 1248 (CO); δ_H(250 MHz; $CDCl_3$) 7.23 (2H, d, *J* 9.0, 2 x H_{ar}), 6.87 (2H, d, *J* 9.0, 2 x H_{ar}), 4.34 (2H, s, $PhCH_2$), 3.80 (3H, s, OCH_3), 3.71 (2H, s, $SnCH_2$), 1.56-0.85 (27H, complex m, Bu_3); δ_C(63 MHz; $CDCl_3$) 158.9 (C_{ar}), 130.9 (C_{ar}), 129.0 (2 x CH_{ar}), 113.5 (2 x CH_{ar}), 76.7 ($PhCH_2$), 61.0 ($SnCH_2$), 55.1 (OCH_3), 29.0 (3 x CH_2 of Bu_3), 27.2 (3 x CH_2 of Bu_3), 13.6 (3 x CH_3 of Bu_3), 8.9 (3 x CH_2 of Bu_3); *m/z* (FAB) 445 (1.1%), 444 (3.9), 443 (2.3), 442 (2.3), 441 (3.5), 440 (4.4), 439 (2.7), 438 (1.0), 437 (1.5), 389 (7.3), 388 (3.8), 387 (18), 386 (11), 385 (34), 384 (32), 383 (37), 382 (12), 381 (26), 121 ($CH_3OC_6H_4CH_2$, 100). Spectroscopic data was consistent with that stated in the literature.[107]

(b) (1*S*,4*S*,5*S*)-4,5-(Isopropylidenedioxy)-1-[(*p*-methoxybenzyloxy)methyl]-cyclopent-2-en-1-ol 125[107]



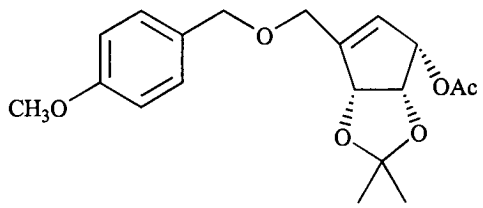
A solution of [(*p*-methoxybenzyloxy)methyl]tri-*n*-butylstannane **108** (8.98 g, 20.4 mmol, 1.0 eq) in anhydrous THF (100 ml) was cooled to -78°C. *n*-BuLi (1.54 M solution in hexane, 13.00 ml, 20.0 mmol, 1.0 eq) was then added dropwise over 2 min so as to maintain the temperature below -70°C and the resulting yellow solution was stirred at -78°C under nitrogen for 5 min. A solution of the enone **61** (3.08 g, 20.0 mmol, 1.0 eq) in anhydrous THF (50 ml) was then added *via* nitrogen pressure transfer through a double tipped needle and the solution was stirred at -78°C for 16 min before being quenched by the addition of saturated aqueous ammonium chloride (100 ml). The mixture was poured into ethyl acetate (350 ml) and the organic layer was separated. The aqueous phase was extracted with ethyl acetate (150 ml) and the combined organic layers were washed with water (300 ml) and brine (300 ml), dried over sodium sulfate, filtered and concentrated. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (3:7) as the eluent to give the *alcohol* **125** as a colourless oil (4.48 g, 73%); R_f 0.69 (EtOAc:hexane, 1:1); $[\alpha]_D^{25}$ +68.7 (*c* 1.10 in $CHCl_3$) lit.[107] $[\alpha]_D^{25}$ +68.7 (*c* 1.20 in CH_2Cl_2); ν_{max}(neat)/cm^{-1} 3513 (OH), 2987, 2935 and 2857 (CH), 1612 (C=C), 1513 ($C{=}C_{ar}$), 1371 (CMe_2), 1246 (CO); δ_H(250 MHz; $CDCl_3$) 7.21 (2H, d, *J* 9.0, 2 x H_{ar}), 6.85 (2H, d, *J* 9.0, 2 x H_{ar}) 5.89 (1H, dd, *J* 1.5 and 6.0, 3-H), 5.73 (1H, d, *J* 6.0, 2-H), 5.01 (1H, dd, *J* 1.5 and 5.5, 4-H), 4.47 (3H, m, $PhCH_2$ and 5-H), 3.79 (3H, s, OCH_3), 3.51 (1H, d, *J* 9.5, CH_2), 3.39 (1H, d, *J* 9.5, CH_2), 3.17 (1H, br s, OH), 1.43 (3H, s, CCH_3), 1.37 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 159.1 (C_{ar}), 136.9 (=CH), 132.8 (=CH), 129.9 (C_{ar}), 129.1 (2 x CH_{ar}), 113.6 (2 x CH_{ar}), 112.4 (CMe_2), 83.7 (CH), 81.6 (C-1), 80.3 (CH), 73.4 (CH_2), 73.1 (CH_2), 55.1 (OCH_3), 27.6 (CH_3), 26.5 (CH_3); *m/z* (EI) 306 (M^+, 1%), 121 ($CH_3OC_6H_4CH_2$, 100); [Found: M^+, 306.14579. $C_{17}H_{22}O_5$ requires M, 306.14672].

(c) (1*S*,4*S*,5*S*)-4,5-(Isopropylidenedioxy)-1-[(*p*-methoxybenzyloxy)methyl]-cyclopent-2-enyl-1-acetate 143



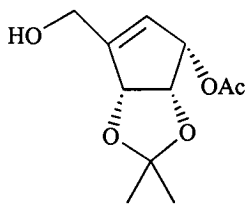
DMAP (177 mg, cat.) was added to a stirred solution of alcohol **125** (1.83 g, 5.97 mmol, 1.0 eq), anhydrous pyridine (3.96 ml, 49.0 mmol, 8.2 eq) and acetic anhydride (3.21 ml, 34.1 mmol, 5.7 eq) in anhydrous dichloromethane (25 ml). After stirring at room temperature under nitrogen for 2 days the reaction mixture was poured into dichloromethane (200 ml) and washed with water (3 x 120 ml). The aqueous portion was extracted with dichloromethane (3 x 150 ml) and the combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. After the removal of excess pyridine by azeotropic distillation with toluene (3 x 100 ml), the residue was purified by flash column chromatography using ethyl acetate-hexane (3:17) as the eluent to give the *ester* **143** as a colourless oil which formed a white solid in the freezer (2.00 g, 96%); mp 57-59°C; (Found: C, 65.27; H, 6.66. $C_{19}H_{24}O_6$ requires C, 65.50; H, 6.94%); R_f 0.72 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ +100.4 (*c* 0.98 in $CHCl_3$) lit.[107] $[\alpha]_D^{25}$ +94.2 (*c* 1.00 in CH_2Cl_2); ν_{max}(nujol)/cm^{-1} 2979 (CH), 1736 (C=O), 1607 (C=C), 1512 (C=C_{ar}), 1377 (CMe_2), 1248 (CO); δ_H(250 MHz; $CDCl_3$) 7.17 (2H, d, *J* 9.0, 2 x H_{ar}), 6.85 (2H, d, *J* 9.0, 2 x H_{ar}), 5.98 (2H, s, 2-H and 3-H), 5.00 (1H, d, *J* 5.5, 4-H), 4.74 (1H, d, *J* 5.5, 5-H), 4.41 (2H, s, $PhCH_2$), 3.81 (1H, d, *J* 9.5, CH_2), 3.79 (3H, s, OCH_3), 3.74 (1H, d, *J* 9.5, CH_2), 2.05 (3H, s, $OC(O)CH_3$), 1.36 (3H, s, CCH_3), 1.34 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 169.9 (C=O), 159.1 (C_{ar}), 134.2 (=CH), 133.4 (=CH), 129.7 (C_{ar}), 129.0 (2 x CH_{ar}), 113.6 (2 x CH_{ar}), 111.9 (CMe_2), 88.7 (C-1), 83.7 (CH), 80.6 (CH), 73.0 (CH_2), 70.9 (CH_2), 55.1 (OCH_3), 27.5 (CH_3), 26.9 (CH_3), 21.5 ($OC(O)CH_3$); *m/z* (FAB) 349 (MH^+, 4%), 121 ($CH_3OC_6H_4CH_2$, 100); [Found: MH^+, 349.16618. $C_{19}H_{24}O_6$ requires MH 349.16511].

(d) (1*S*,2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(*p*-methoxybenzyloxy)methyl]-cyclopent-4-enyl-1-acetate 144



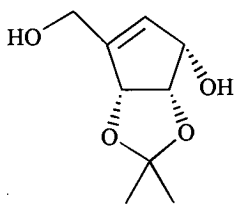
To a solution of the ester **143** (475 mg, 1.36 mmol, 1.0 eq) in anhydrous THF (27 ml) was added bis(acetonitrile)dichloropalladium(II) complex (38 mg, 0.15 mmol, 11 mol %) and benzoquinone (62 mg, 0.58 mmol, 0.4 eq). The resulting orange solution was heated at reflux under nitrogen for 4 h 15 min. After cooling to room temperature the mixture was concentrated under reduced pressure. The resulting brown oil was purified by flash column chromatography using ethyl acetate-hexane (1:3) as the eluent to give the *acetate* **144** as a yellow oil (299 mg, 63%); R_f 0.67 (EtOAc:hexane, 1:1); ν_{max}(neat)/cm^{-1} 3018 (C=CH), 2959 (CH), 1736 (C=O), 1513 (C=C_{ar}), 1379 (CMe_2), 1218 (CO); δ_H(250 MHz; $CDCl_3$) 7.26 (2H, d, *J* 9.0, 2 x H_{ar}), 6.87 (2H, d, *J* 9.0, 2 x H_{ar}), 5.76 (1H, d, *J* 2.0, 6-H), 5.34 (1H, m, 1-H), 4.91 (2H, m, 2-H and 3-H), 4.49 (2H, s, $PhCH_2$), 4.15 (2H, m, CH_2), 3.79 (3H, s, OCH_3), 2.10 (3H, s, $OC(O)CH_3$), 1.38 (3H, s, CCH_3), 1.36 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 170.5 (C=O), 159.1 (C_{ar}), 145.6 (C=), 129.8 (C_{ar}), 129.2 (2 x CH_{ar}), 126.2 (=CH), 113.7 (2 x CH_{ar}), 112.8 (CMe_2), 82.7 (CH), 77.1 (CH), 75.1 (CH), 72.6 (CH_2), 66.1 (CH_2), 55.2 (OCH_3), 27.2 (CH_3), 26.5 (CH_3), 20.7 ($OC(O)CH_3$). ^{1}H and ^{13}C NMR spectra also showed minor peaks due to an impurity. The acetate was not further purified but was carried on to the next reaction.

(e) (1*S*,2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-hydroxymethylcyclopent-4-enyl-1-acetate 148[107]



DDQ (121 mg, 0.53 mmol, 1.5 eq) was added to a solution of the acetate **144** (124 mg, 0.36 mmol, 1.0 eq) in CH_2Cl_2:H_2O (20:1) (8 ml) at 0°C. The mixture was allowed too warm to room temperature and after stirring for 10 min a pink solid precipitated. Stirring was continued for 2 h 30 min after which time saturated aqueous sodium bicarbonate (10 ml) was added and the mixture was extracted with dichloromethane (3 x 20 ml). The organic portion was washed with water (30 ml) and brine (30 ml). The aqueous portions were further extracted with dichloromethane (2 x 30 ml) and the combined organic portions were dried over sodium sulfate, filtered and concentrated. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (1:1) as the eluent to give the *acetate* **148** as a pale yellow oil (68 mg, 84%); R_f 0.23 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ –55.1 (*c* 1.15 in $CHCl_3$) lit.[107] $[\alpha]_D^{25}$ –55.5 (*c* 1.30 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3459 (OH), 2989 and 2938 (CH), 1732 (C=O), 1372 (CMe_2), 1238 (CO); δ_H(250 MHz; $CDCl_3$) 5.72 (1H, d, *J* 2.0, 6-H), 5.35 (1H, m, 1-H), 4.94 (2H, m, 2-H and 3-H), 4.34 (2H, m, CH_2), 2.10 (3H, s, $OC(O)CH_3$), 1.39 (3H, s, CCH_3), 1.35 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 170.5 (C=O), 147.5 (C=), 125.3 (=CH), 112.9 (CMe_2), 82.9 (CH), 77.2 (CH), 75.0 (CH), 59.9 (CH_2), 27.1 (CH_3), 26.4 (CH_3), 20.7 ($OC(O)CH_3$); *m/z* (FAB) 229 (MH^+, 24%); [Found: MH^+, 229.10765. $C_{11}H_{16}O_5$ requires 229.10760].

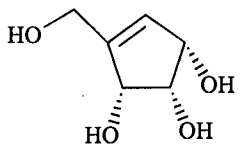
(f) (1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-hydroxymethylcyclopent-4-en-1-ol 129



A solution of the acetate **148** (166 mg, 0.73 mmol) in ammonia saturated methanol (23 ml) was stirred at room temperature for 19 h 30 min before being concentrated under reduced pressure. The resulting brown oil was then purified by flash column chromatography using ethyl acetate-hexane (1:1) (150 ml), ethyl acetate-hexane (3:1) (250 ml) and then ethyl acetate as the eluent to give the *protected diol* **129** as a pale yellow oil (130 mg, 96%); R_f 0.37 (EtOAc); $[\alpha]_D^{24}$ +41.0

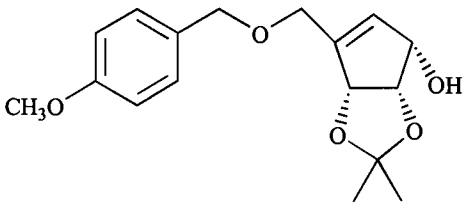
(*c* 1.20 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3402 (OH), 2987 and 2934 (CH), 1650 (C=C), 1374 (CMe_2), 1238 (CO); δ_H(250 MHz; $CDCl_3$) 5.72 (1H, d, *J* 1.5, 6-H), 4.96 (1H, d, *J* 5.5, 3-H), 4.76 (1H, t, *J* 5.5, 2-H), 4.55 (1H, m, 1-H), 4.28 (2H, m, CH_2), 2.76 (1H, br d, OH), 2.24 (1H, br s, OH), 1.42 (3H, s, CCH_3), 1.38 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 144.4 (C=), 130.1 (=CH), 112.5 (CMe_2), 83.0 (CH), 77.7 (CH), 73.1 (CH), 59.6 (CH_2), 27.4 (CH_3), 26.3 (CH_3); *m/z* (FAB) 187 (MH^+, 10%); [Found: MH^+, 187.09765. $C_9H_{14}O_4$ requires 187.09703].

(g) (1*S*,2*S*,3*R*)-4-Hydroxymethylcyclopent-4-en-1,2,3-triol 83a



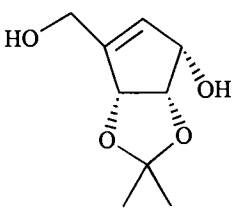
To a solution of the protected diol **129** (111 mg, 0.60 mmol) in THF:H_2O (4:1) (3 ml) at 0°C was added trifluoroacetic acid (0.25 ml). The cooling bath was removed and the pale yellow solution was stirred at room temperature for 16 h after which time TLC showed that starting material remained, therefore additional trifluoroacetic acid (0.1 ml) was added. The solution was stirred for a further 15 h before being concentrated under reduced pressure. The resulting brown oil was purified by flash column chromatography using methanol-chloroform (3:7) as the eluent to give the *tetrol* **83a** as a colourless oil (82 mg, 94%); R_f 0.23 (MeOH:$CHCl_3$, 1:4); $[\alpha]_D^{24}$ +23.7 (*c* 0.84 in H_2O); ν_{max}(neat)/cm^{-1} 3369 (OH), 2930 (CH), 1677 (C=C), 1203 (CO); δ_H(250 MHz; D_2O) 5.83 (1H, m, 6-H), 4.44 (1H, m, 1-H), 4.38 (1H, d, *J* 5.5, 3-H), 4.22 (2H, m, CH_2), 4.11 (1H, t, *J* 5.5, 2-H); δ_C(63 MHz; D_2O) 147.2 (C=), 127.9 (=CH), 72.8 (CH), 72.5 (CH), 71.8 (CH), 58.4 (CH_2); *m/z* (FAB) 147 (MH^+, 12%); [Found: MH^+, 147.06561. $C_6H_{10}O_4$ requires 147.06573].

(h) (1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(*p*-methoxybenzyloxy)methyl]-cyclopent-4-en-1-ol 145



To a solution of the acetate **144** (299 mg, 0.86 mmol, 1.0 eq) in anhydrous methanol (38 ml) was added anhydrous potassium carbonate (498 mg, 3.60 mmol, 4.2 eq, excess). After stirring at room temperature under nitrogen for 1 h the mixture was diluted with ethyl acetate (30 ml) and filtered through a pad of silica. The filtrate was then concentrated under reduced pressure. The resulting brown oil was purified by flash column chromatography using ethyl acetate-hexane (7:13) as the eluent to give the *PMB-protected alcohol* **145** as a yellow oil (160 mg, 61%); R_f 0.46 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ +34.1 (*c* 1.10 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3449 (OH), 3034 (C=CH), 2933 (CH), 1612 (C=C), 1513 (C=C_{ar}), 1380 (CMe_2), 1247 (CO); δ_H(250 MHz; $CDCl_3$) 7.26 (2H, d, *J* 9.0, 2 x H_{ar}), 6.86 (2H, d, *J* 9.0, 2 x H_{ar}), 5.77 (1H, d, *J* 1.5, 6-H), 4.95 (1H, d, *J* 5.5, 3-H), 4.74 (1H, t, *J* 5.5, 2-H), 4.52 (1H, m, 1-H), 4.47 (2H, s, $PhCH_2$), 4.10 (2H, m, CH_2), 3.79 (3H, s, OCH_3), 2.70 (1H, br d, OH), 1.40 (3H, s, CCH_3), 1.39 (3H, s, CCH_3); δ_C(63 MHz; $CDCl_3$) 159.1 (C_{ar}), 142.5 (C=), 131.2 (=CH), 129.9 (C_{ar}), 129.2 (2 x CH_{ar}), 113.7 (2 x CH_{ar}), 112.4 (CMe_2), 82.9 (CH), 77.5 (CH), 73.2 (CH), 72.5 (CH_2), 65.8 (CH_2), 55.2 (OCH_3), 27.5 (CH_3), 26.5 (CH_3); *m/z* (FAB) 307 (MH^+, 2%), 121 ($CH_3OC_6H_4CH_2$, 100); [Found: MH^+, 307.15433. $C_{17}H_{22}O_5$ requires MH, 307.15455].

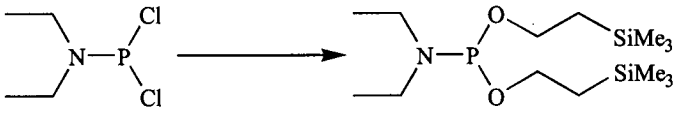
(i) (1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-hydroxymethylcyclopent-4-en-1-ol 129



DDQ (177 mg, 0.78 mmol, 1.5 eq) was added to a solution of the PMB-protected alcohol **145** (159 mg, 0.52 mmol, 1.0 eq) in CH_2Cl_2:H_2O (20:1) (12 ml) at 0°C. The cooling bath was removed and the mixture was stirred at room temperature for 3 h 30 min before saturated aqueous sodium bicarbonate (20 ml) was added and the mixture was extracted with dichloromethane (4 x 30 ml). The organic portion was washed with saturated aqueous sodium bicarbonate (20 ml), water (30 ml) and brine (30 ml). The aqueous portions were saturated with sodium chloride and further extracted with ethyl acetate (5 x 75 ml) and the combined organic portions were dried over sodium sulfate, filtered and concentrated. The resulting yellow oil was purified by flash column chromatography using ethyl acetate as the eluent to give the *protected diol* **129** as a pale yellow oil (37 mg, 38%). Spectroscopic data was consistent with that for the previous procedure.

5.3.3 Synthesis of phosphorylated compounds

(a) Bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite 150[107]

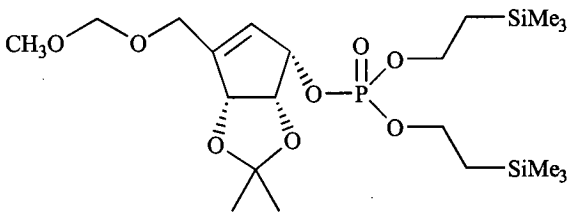


Diethylamine (20.70 ml, 0.20 mol, 2.0 eq) was added dropwise to a solution of phosphorous trichloride (8.70 ml, 0.10 mol, 1.0 eq) in anhydrous THF (60 ml) cooled to -40°C. The cooling bath was replaced with an ice bath and after stirring for 30 min the ice bath was removed and the resulting thick yellow mixture was stirred at room temperature for 4 h. The yellow mixture was filtered through an oven-dried sintered funnel and the solid was washed with anhydrous THF (2 x 50 ml). The filtrate was then concentrated under reduced pressure to give a pale yellow liquid. Distillation at 16 mbar (68°C) gave the N,N-*diethylphosphorochloridite* **149** as a colourless liquid (9.11 g, 53%).

A solution of 2-(trimethylsilyl)ethanol (6.05 ml, 42.2 mmol, 2.0 eq) and triethylamine (6.50 ml, 46.6 mmol, 2.2 eq) in anhydrous THF (15 ml) at 0°C, was added *via* nitrogen pressure transfer through a double tipped needle to a solution of *N*,*N*-diethylphosphorochloridite **149** (3.68 g, 21.1 mmol, 1.0 eq) in anhydrous THF (10 ml) cooled to 0°C. The cooling bath was removed and the mixture was stirred at room temperature for 4 h. Aqueous sodium bicarbonate (5% w/v, 50 ml) was then added followed by ethyl acetate (100 ml). The organic layer was washed with aqueous sodium bicarbonate (5% w/v, 2 x 50 ml) and brine (75 ml), dried over sodium sulfate, filtered and concentrated to give a pale yellow liquid. Distillation at 1.0 mmHg (120°C) (lit.[107] bp 119-122°C at 3.0 mmHg) gave the *phosphoramidite* **150** as a colourless liquid (2.90 g, 41% from *N*,*N*-diethyl-phosphoramidous dichloride); R_f 0.89 ($MeOH:CHCl_3$, 1:9); ν_{max}(neat)/cm^{-1} 2954, 2896 and 2867 (CH), 1249 ($SiMe_3$), 1202 (CO), 1043 (P-O-alkyl), 838 ($SiMe_3$); δ_H(250 MHz; $CDCl_3$) 3.78-3.58 (4H, m, 2 x OCH_2), 3.09-2.97 (4H, dq, J_{HH} 7.0 and J_{PH} 9.5, 2 x CH_2N), 1.02 (6H, t, *J* 7.0, 2 x CH_3CH_2N), 1.01-0.94 (4H, m, 2 x CH_2Si), -0.01 (18H, s, 2 x $SiMe_3$); δ_C(63 MHz; $CDCl_3$) 60.4 ($2CH_2$, d, J_{PC} 16.5, 2 x OCH_2), 37.3 ($2CH_2$, d, J_{PC}

20.5, 2 x CH_2N), 19.9 ($2CH_2$, d, J_{PC} 6.0, 2 x CH_2Si), 14.9 ($2CH_3$, d, J_{PC} 3.0, 2 x CH_3CH_2N), -1.5 ($6CH_3$, 2 x $SiMe_3$); δ_P(101 MHz; $CDCl_3$) 145.65; *m/z* (FAB) 338 (MH^+, 4%), 192 (MH^+-$(SiMe_3)_2$, 82), 73 ($SiMe_3$, 100); [Found: MH^+, 338.20950. $C_{14}H_{36}NO_2PSi_2$ requires MH, 338.21005].

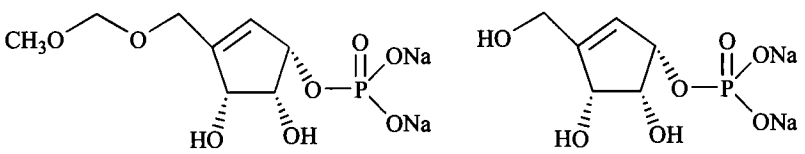
(b) (1*S*,2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(methoxymethoxy)methyl]-1-bis[2-(trimethylsilyl)ethyl]-cyclopent-4-enyl-1-phosphate 151



A solution of alcohol **130** (78 mg, 0.34 mmol, 1.0 eq) in anhydrous THF (0.8 ml) was added dropwise *via* syringe to a stirred solution of 1*H*-tetrazole (81 mg, 1.16 mmol, 3.4 eq) and bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150** (140 mg, 0.41 mmol, 1.2 eq) in anhydrous THF (3 ml). The reaction mixture was stirred at room temperature for 1 h 10 min before being cooled to -40°C. A solution of MCPBA (68 mg, 0.39 mmol, 1.2 eq) in anhydrous dichloromethane (0.8 ml) was then added *via* nitrogen pressure transfer through a double tipped needle and the mixture was stirred at -40°C for 30 min. The cooling bath was removed and the mixture was allowed to warm to room temperature before being stirred at room temperature for 1 h. Diethyl ether (40 ml) was added and the solution was washed with aqueous sodium bisulfite (10% w/v, 2 x 20 ml) and aqueous sodium bicarbonate (5% w/v, 2 x 20 ml). The aqueous portions were further extracted with diethyl ether (30 ml) and the combined organic layers were dried over sodium sulfate, filtered and concentrated. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (1:3) + triethylamine (5 ml in 1 litre) as the eluent to give the *fully protected phosphate* **151** as a pale yellow oil (95 mg, 55%); R_f 0.60 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ +6.8 (*c* 0.90 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 2955 (CH), 1641 (C=C), 1379 (CMe_2), 1262 ($SiMe_3$), 1251 (P=O), 1212 (CO), 1052 (P-O-alkyl), 838 ($SiMe_3$); δ_H(250 MHz; $CDCl_3$) 5.79 (1H, m, 6-H), 5.15 (1H, m, 1-H), 4.90 (1H, d, *J* 5.0, 3-H), 4.84 (1H, t, *J* 5.0, 2-H), 4.64 (2H, s, OCH_2O), 4.24-4.11

(6H, m, CH_2 and 2 x POCH_2CH_2Si), 3.36 (3H, s, OCH_3), 1.38 (3H, s, CCH_3), 1.36 (3H, s, CCH_3), 1.26-1.06 (4H, m, 2 x CH_2Si), 0.02 (18H, s, 2 x $SiMe_3$); δ_C(63 MHz; $CDCl_3$) 144.6 (C=), 127.1 (1CH, d, J_{PC} 6.5, =CH), 112.7 (CMe_2), 96.0 (OCH_2O), 82.6 (C-3H), 77.7 (1CH, d, J_{PC} 3.0, C-2H), 77.3 (1CH, d, J_{PC} 5.5, C-1H), 66.3 (1CH_2, d, J_{PC} 6.5, POCH_2CH_2Si), 66.2 (1CH_2, d, J_{PC} 6.5, POCH_2CH_2Si), 63.1 (CH_2), 55.2 (CH_3O), 27.5 (CH_3), 26.8 (CH_3), 19.4 (1CH_2, d, J_{PC} 5.0, CH_2Si), 19.3 (1CH_2, d, J_{PC} 5.0, CH_2Si), -1.6 (6CH_3, 2 x $SiMe_3$); δ_P(101 MHz; $CDCl_3$) –1.10; *m/z* (FAB) 511 (MH^+, 1%), 1022 (MH^+ dimer, 7), 73 ($SiMe_3$, 100), 45 (CH_3OCH_2, 48); [Found: MH^+, 511.23321. $C_{21}H_{43}O_8PSi_2$ requires MH, 511.23124].

(c) Attempted deprotection of (1*S*,2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(methoxymethoxy)methyl]-1-bis[2-(trimethylsilyl)ethyl]-cyclopent-4-enyl-1-phosphate 151



The fully protected phosphate **151** (72 mg, 0.14 mmol) was placed in a Teflon bottle. Acetonitrile (10.9 ml) was added followed by deionized water (1.9 ml) and the colourless solution was cooled to 0°C. Hydrofluoric acid (48-51% aqueous solution, 1.3 ml) was added and the ice bath was removed. The solution was then stirred at room temperature for 24 h after which time methoxytrimethylsilane (10 ml) was added and the mixture was stirred for 10 min before being transferred to a round-bottomed flask and concentrated under reduced pressure. Further methoxytrimethylsilane (10 ml) was added and the solution was again concentrated. Deionized water (5 ml) was added to the resulting yellow glass and the solution was filtered through a plug of cotton wool, the filtrate being concentrated to give the *phosphate (free acid)* as a pale yellow glass (39 mg).

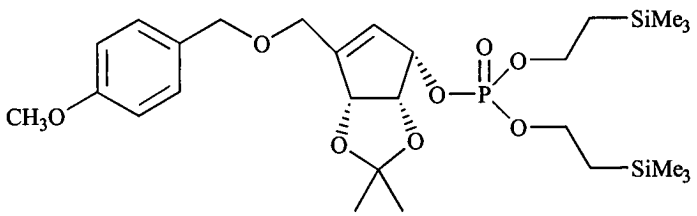
1 M NaOH (approximately 0.2 ml) was added dropwise to a solution of the phosphate (free acid) (39 mg) in deionized water (2 ml), until a pH = 8 was obtained. The pale yellow solution was concentrated under reduced pressure to give a yellow

solid that was washed repeatedly with ethyl acetate and then dried under vacuum to give a pale yellow solid (39 mg). ^{1}H, ^{13}C, ^{31}P NMR and mass spectrometry appeared to show that a mixture of the desired *fully deprotected phosphate (disodium salt)* **152** and the *MOM-protected phosphate (disodium salt)* **153** had been formed. However, the NMR data was complex and difficult to assign unambiguously.

(1S,2R,3R)-4-Hydroxymethyl-2,3-dihydroxy-cyclopent-4-enyl-1-phosphate (disodium salt) **152**; R_f 0.57 (MeCN:0.1 M NH_4Cl, 6:4); *m/z* (ES-) 269.1 ($(M-H)^-$, 82%), 225.0 (MH-2Na, 41).

(1S,2R,3R)-4-[(Methoxymethoxy)methyl]-2,3-dihydroxy-cyclopent-4-enyl-1-phosphate (disodium salt) **153**; R_f 0.66 (MeCN:0.1 M NH_4Cl, 6:4); m/z (ES-) 313.1 ($(M-H)^-$, 86%).

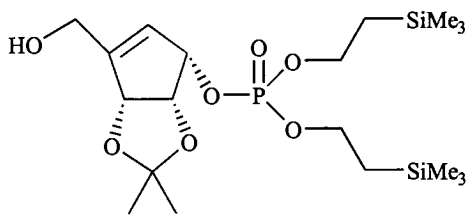
(d) (1*S*,2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(*p*-methoxybenzyloxy)methyl]-1-bis[2-(trimethylsilyl)ethyl]-cyclopent-4-enyl-1-phosphate 154



A solution of alcohol **145** (99 mg, 0.32 mmol, 1.0 eq) in anhydrous THF (1 ml) was added dropwise *via* syringe to a stirred solution of 1*H*-tetrazole (88 mg, 1.26 mmol, 3.9 eq) and bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150** (156 mg, 0.46 mmol, 1.4 eq) in anhydrous THF (4 ml). The reaction mixture was then stirred at room temperature under nitrogen for 1 h 40 min before being cooled to -70°C. A solution of MCPBA (69 mg, 0.40 mmol, 1.2 eq) in anhydrous dichloromethane (1 ml) was added *via* syringe and the mixture was stirred at -70°C for 30 min. The cooling bath was then removed and the mixture was allowed to warm to room temperature before being stirred at room temperature for a further 1 h 30 min. Diethyl ether (40 ml) was added and the solution was washed with aqueous sodium bisulfite (10% w/v, 2 x 20 ml) and aqueous sodium bicarbonate (5% w/v, 2 x

20 ml). The aqueous portions were further extracted with diethyl ether (2 x 50 ml) and the combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (1:3) as the eluent to give the *fully protected phosphate* **154** as a pale yellow oil (157 mg, 83%); R_f 0.47 (EtOAc:hexane, 1:1); $[\alpha]_D^{26}$ +7.5 (*c* 1.02 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 2953 and 2902 (CH), 1613 (C=C), 1514 (C=C_{ar}), 1379 (CMe_2), 1250 (P=O), 1211 (CO), 1065 (P-O-alkyl), 857 ($SiMe_3$); δ_H(250 MHz; $CDCl_3$) 7.25 (2H, d, *J* 8.5, 2 x H_{ar}), 6.86 (2H, d, *J* 8.5, 2 x H_{ar}), 5.80 (1H, s, 6-H), 5.14 (1H, m, 1-H), 4.90 (1H, d, *J* 5.5, 3-H), 4.83 (1H, t, *J* 5.5, 2-H), 4.48 (2H, s, $PhCH_2$), 4.25-4.11 (6H, m, CH_2 and 2 x $POCH_2CH_2Si$), 3.79 (3H, s, OCH_3), 1.38 (3H, s, CCH_3), 1.36 (3H, s, CCH_3), 1.27-1.06 (4H, m, 2 x CH_2Si), 0.02 (18H, s, 2 x $SiMe_3$); δ_C(63 MHz; $CDCl_3$) 159.1 (C_{ar}), 144.9 (C=), 129.8 (C_{ar}), 129.2 (2 x CH_{ar}), 127.2 (1CH, d, J_{PC} 6.5, =CH), 113.7 (2 x CH_{ar}), 112.7 (CMe_2), 82.6 (C-3H), 77.7 (1CH, d, J_{PC} 3.5, C-2H), 77.2 (1CH, d, J_{PC} 5.5, C-1H), 72.5 ($PhCH_2$), 66.3 ($1CH_2$, d, J_{PC} 7.0, $POCH_2CH_2Si$), 66.2 ($1CH_2$, d, J_{PC} 7.0, $POCH_2CH_2Si$), 65.7 (CH_2), 55.2 (OCH_3), 27.5 (CH_3), 26.8 (CH_3), 19.4 ($1CH_2$, d, J_{PC} 5.0, CH_2Si), 19.3 ($1CH_2$, d, J_{PC} 5.0, CH_2Si), -1.6 ($6CH_3$, 2 x $SiMe_3$); δ_P(101 MHz; $CDCl_3$) –1.08; *m/z* (FAB) 587 (MH^+, 1%), 1174 (MH^+ dimer, 2), 121 ($CH_3OC_6H_4CH_2$, 100), 73 ($SiMe_3$, 89); [Found: MH^+, 587.26318. $C_{27}H_{47}O_8PSi_2$ requires MH, 587.26254].

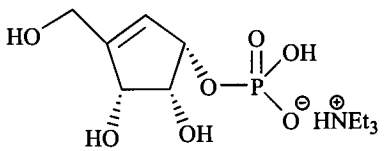
(e) (1*S*,2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-hydroxymethyl-1-bis[2-(trimethylsilyl)ethyl]-cyclopent-4-enyl-1-phosphate 155



DDQ (52 mg, 0.23 mmol, 1.5 eq) was added to a solution of the fully protected phosphate **154** (87 mg, 0.15 mmol, 1.0 eq) in CH_2Cl_2:H_2O (20:1) (4 ml) cooled to 0°C. The cooling bath was removed and the mixture was allowed to warm to room temperature before being stirred at room temperature for 4 h 30 min. Saturated

aqueous sodium bicarbonate (15 ml) was added and the mixture was extracted with dichloromethane (3 x 25 ml). The combined organic portions were washed with water (40 ml) and brine (30 ml), dried over sodium sulfate, filtered and concentrated. The resulting yellow oil was purified by flash column chromatography using ethyl acetate-hexane (4:1) as the eluent to give the *alcohol* **155** as a colourless oil (65 mg, 94 %); R_f 0.38 (EtOAc:hexane, 9:1); $[\alpha]_D^{24}$ +5.4 (*c* 1.09 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3400 (OH), 2953 and 2901 (CH), 1664 (C=C), 1379 (CMe_2), 1250 (P=O), 1212 (CO), 1060 (P-O-alkyl), 856 ($SiMe_3$); δ_H(250 MHz; $CDCl_3$) 5.76 (1H, m, 6-H), 5.14 (1H, m, 1-H), 4.93 (1H, d, *J* 5.5, 3-H), 4.85 (1H, t, *J* 5.5, 2-H), 4.42-4.25 (2H, m, CH_2), 4.24-4.05 (4H, m, 2 x POC*H*$_2$$CH_2Si$), 2.03 (1H, br s, OH), 1.40 (3H, s, CCH_3), 1.36 (3H, s, CCH_3), 1.14-1.06 (4H, m, 2 x CH_2Si), 0.02 (18H, s, 2 x $SiMe_3$); δ_C(63 MHz; $CDCl_3$) 146.8 (C=), 126.2 (1CH, d, J_{PC} 7.0, =CH), 112.8 (CMe_2), 82.8 (C-3H), 77.8 (1CH, d, J_{PC} 3.0, C-2H), 77.3 (1CH, d, J_{PC} 5.5, C-1H), 66.4 ($1CH_2$, d, J_{PC} 7.0, POC*H*$_2$$CH_2Si$), 66.3 ($1CH_2$, d, J_{PC} 7.0, POC*H*$_2$$CH_2Si$), 59.6 ($CH_2$), 27.5 ($CH_3$), 26.6 ($CH_3$), 19.4 ($1CH_2$, d, J_{PC} 5.0, CH_2Si), 19.3 ($1CH_2$, d, J_{PC} 5.0, CH_2Si), -1.6 ($6CH_3$, 2 x $SiMe_3$); δ_P(101 MHz; $CDCl_3$) –1.13; *m/z* (FAB) 467 (MH^+, 3%), 934 (MH^+ dimer, 4), 73 ($SiMe_3$, 100); [Found: MH^+, 467.20593. $C_{19}H_{39}O_7PSi_2$ requires MH, 467.20503].

(f) (1*S*,2*R*,3*R*)-4-Hydroxymethyl-2,3-dihydroxy-cyclopent-4-enyl-1-phosphate (triethylammonium salt) 152



<u>*Via* the disodium salt</u>

A solution of alcohol **155** (46 mg, 0.10 mmol), acetonitrile (7.6 ml) and deionized water (1.3 ml) in a Teflon bottle was cooled to 0°C. Hydrofluoric acid (48-51% aqueous solution, 0.9 ml) was then added, the cooling bath was removed and the mixture was stirred at room temperature for 6 h after which time additional HF (0.5 ml) was added and the mixture was stirred for a further 1 h. Approximately half of the solvent was evaporated by passing nitrogen over the solution then

methoxytrimethylsilane (10 ml) was added and the mixture was stirred at room temperature for 10 min before being transferred to a round-bottomed flask and concentrated under reduced pressure. Further methoxytrimethylsilane (8 ml) was added to the concentrated residue, which was again concentrated to give the *phosphate (free acid)* as an off-white solid (16 mg).

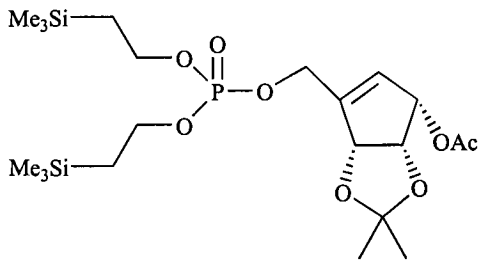
The phosphate (free acid) (16 mg) was dissolved in the minimum volume of deionized water (approximately 2 ml) and 0.1 M NaOH (approximately 3 ml) was added dropwise until a pH = 8 was obtained. The solution was then concentrated to give a pale yellow glass. Methanol:acetone (1:1) (2 ml) was added and the glass was scratched with a spatula at which point an off-white solid crashed out. The solid was washed with diethyl ether (2 x 5 ml) before being dried under vacuum to give an off-white solid (26 mg). ^{1}H, ^{13}C, ^{31}P NMR and mass spectrometry showed that the product was a mixture of the fully deprotected phosphate and the phosphate with one (trimethylsilyl)ethyl group still attached.

The phosphate mixture (20 mg) was purified by ion-exchange chromatography using triethylammonium bicarbonate buffer solution to give the desired *fully deprotected phosphate* **152** as a pale yellow glass (18 mg, 60%) and *the (trimethylsilyl)ethyl-protected phosphate* **156** as a colourless glass (1 mg). The 10 mM and 15 mM portions of the buffer solution eluted the trimethylsilylethyl-protected phosphate and the 20, 25, 30, 35 and 40 mM portions eluted the fully deprotected phosphate.

(1S,2R,3R)-4-Hydroxymethyl-2,3-dihydroxy-cyclopent-4-enyl-1-phosphate (triethylammonium salt) **152**; R_f 0.38 (MeCN:0.1 M NH_4Cl, 6:4); $[\alpha]_D^{24}$ +16.6 (*c* 0.82 in H_2O); ν_{max}(neat)/cm^{-1} 3399 (OH), 2985 (CH), 1651 (C=C), 1250 (P=O), 1214 (CO), 1055 (P-O-alkyl); δ_H(250 MHz; D_2O) 5.95 (1H, d, *J* 1.5, 6-H), 4.41 (1H, d, *J* 5.5, 3-H), 4.31-4.21 (4H, m, CH_2, 1-H and 2-H), 3.17 (6H, q, *J* 7.5, 3 x NCH_2), 1.25 (9H, t, *J* 7.5, 3 x NCH_2CH_3); δ_C(91 MHz; D_2O) 148.6 (C=), 126.9 (1CH, d, J_{PC} 3.0, =CH), 76.7 (1CH, d, J_{PC} 5.5, C-2H), 72.9 (C-3H), 72.1 (1CH, d, J_{PC} 5.5, C-1H), 58.8 (CH_2), 47.0 (3 x NCH_2), 8.6 (3 x NCH_2CH_3); δ_P(101 MHz; D_2O) 0.89; *m/z* (FAB)

328 (MH^+, 24%), 429 ($M+CH_3CH_2N$, 7), 102 (Et_3NH, 100); [Found: MH^+, 328.15283. $C_6H_{10}O_7P.C_6H_{16}N$ requires MH, 328.15252].

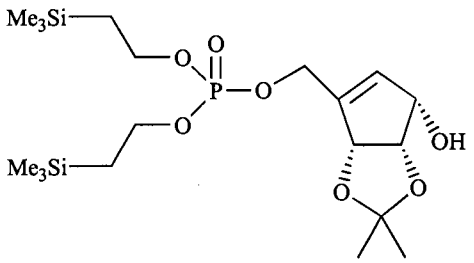
(g) (1*S*,2*R*,3*R*)-2,3-(Isopropylidenedioxy)-4-bis[2-(trimethylsilyl)ethyl]-phosphoryloxymethyl-cyclopent-4-enyl-1-acetate 157



A solution of alcohol **148** (61 mg, 0.27 mmol, 1.0 eq) in anhydrous THF (0.8 ml) was added dropwise *via* syringe to a stirred solution of 1*H*-tetrazole (68 mg, 0.97 mmol, 3.6 eq) and bis[2-(trimethylsilyl)ethyl]-*N*,*N*-diethylphosphoramidite **150** (133 mg, 0.39 mmol, 1.5 eq) in anhydrous THF (3 ml). The reaction mixture was stirred at room temperature for 1 h 35 min before being cooled to -60°C. A solution of MCPBA (56 mg, 0.33 mmol, 1.2 eq) in anhydrous dichloromethane (0.8 ml) was then added *via* nitrogen pressure transfer through a double tipped needle and the mixture was stirred at -60°C for 30 min. The cooling bath was removed and the mixture was allowed to warm to room temperature before being stirred at room temperature for a further 1 h. Diethyl ether (40 ml) was added and the solution was washed with aqueous sodium bisulfite (10% w/v, 2 x 20 ml) and aqueous sodium bicarbonate (5% w/v, 2 x 20 ml). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting pale yellow oil was purified by flash column chromatography using ethyl acetate-hexane (1:3) + triethylamine (6 ml in 1 litre) as the eluent to give the *acetate* **157** as a pale yellow oil (116 mg, 85%); R_f 0.53 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ –18.5 (*c* 1.30 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 2952 and 2900 (CH), 1740 (C=O), 1644 (C=C), 1371 (CMe_2), 1265 ($SiMe_3$), 1249 (P=O), 1179 (CO), 1050 (P-O-alkyl), 857 ($SiMe_3$); δ_H(250 MHz; $CDCl_3$) 5.81 (1H, d, *J* 2.0, 6-H), 5.36 (1H, m, 1-H), 4.93 (2H, m, 2-H and 3-H), 4.68 (2H, m, CH_2), 4.20-4.09 (4H, m, 2 x $POCH_2CH_2Si$), 2.09 (3H, s, $OC(O)CH_3$), 1.36 (3H, s, CCH_3), 1.35 (3H, s, CCH_3), 1.12-1.05 (4H, m, 2 x CH_2Si),

0.02 (18H, s, 2 x $SiMe_3$); δ_C(63 MHz; $CDCl_3$) 170.4 (C=O), 143.6 (1C, d, J_{PC} 7.5, C=), 127.0 (=CH), 112.9 (CMe_2), 82.2 (CH), 77.3 (CH), 74.9 (CH), 66.3 ($2CH_2$, d, J_{PC} 6.5, 2 x $POCH_2CH_2Si$), 63.1 ($1CH_2$, d, J_{PC} 5.0, CH_2), 27.2 (CH_3), 26.6 (CH_3), 20.6 ($OC(O)CH_3$) 19.4 ($2CH_2$, d, J_{PC} 5.5, 2 x CH_2Si), -1.6 ($6CH_3$, 2 x $SiMe_3$); δ_P(101 MHz; $CDCl_3$) –0.48; *m/z* (FAB) 509 (MH^+, 2%), 1018 (MH^+ dimer, 1), 73 ($SiMe_3$, 100), 43 (CH_3CO, 65); [Found: MH^+, 509.21574. $C_{21}H_{41}O_8PSi_2$ requires MH, 509.21559]. ^1H and ^{13}C NMR data was consistent with that stated in the literature.[107]

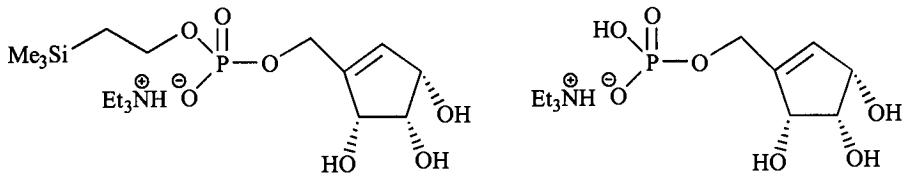
(h) (1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-bis[2-(trimethylsilyl)ethyl]-phosphoryloxymethyl-cyclopent-4-en-1-ol 158



A solution of the acetate **157** (109 mg, 0.21 mmol) in ammonia saturated methanol (7 ml) was stirred at room temperature for 8 h 30 min before being concentrated under reduced pressure. The resulting brown oil was purified by flash column chromatography using ethyl acetate-hexane (1:1) + triethylamine (6 ml in 1 litre) as the eluent to give the *alcohol* **X** as a colourless oil (73 mg, 73%); R_f 0.35 (EtOAc:hexane, 1:1); $[\alpha]_D^{24}$ +26.6 (*c* 1.01 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 3406 (OH), 2954 and 2901 (CH), 1656 (C=C), 1381 (CMe_2), 1269 ($SiMe_3$), 1251 (P=O), 1213 (CO), 1049 (P-O-alkyl), 858 ($SiMe_3$); δ_H(200 MHz; $CDCl_3$) 5.81 (1H, m, 6-H), 4.95 (1H, d, *J* 5.5, 3-H), 4.76 (1H, t, *J* 5.5, 2-H), 4.66-4.54 (3H, m, CH_2 and 1-H), 4.21-4.08 (4H, m, 2 x $POCH_2CH_2Si$), 2.71 (1H, br d, OH), 1.40 (3H, s, CCH_3), 1.38 (3H, s, CCH_3), 1.13-1.04 (4H, m, 2 x CH_2Si), 0.02 (18H, s, 2 x $SiMe_3$); δ_C(63 MHz; $CDCl_3$) 140.7 (1C, d, J_{PC} 7.5, C=), 132.0 (=CH), 112.6 (CMe_2), 82.3 (CH), 77.8 (CH), 73.1 (CH), 66.3 ($2CH_2$, d, J_{PC} 6.0, 2 x $POCH_2CH_2Si$), 63.0 ($1CH_2$, d, J_{PC} 5.0, CH_2), 27.5 (CH_3), 26.5 (CH_3), 19.5 ($2CH_2$, d, J_{PC} 5.5, 2 x CH_2Si), -1.6 ($6CH_3$, 2 x $SiMe_3$); δ_P(81 MHz; $CDCl_3$) –0.40; *m/z* (FAB) 467 (MH^+, 2%), 934 (MH^+ dimer, 2),

73 ($SiMe_3$, 100); [Found: MH^+, 467.20659. $C_{19}H_{39}O_7PSi_2$ requires MH, 467.20503]. ^1H and ^{13}C NMR data was consistent with that stated in the literature.[107]

(i) Deprotection of (1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-bis[2-(trimethylsilyl)ethyl]-phosphoryloxymethyl-cyclopent-4-en-1-ol 158



Via the cyclohexylammonium salt

A stirred solution of alcohol **158** (49 mg, 0.11 mmol) in acetonitrile (8.5 ml) and deionized water (1.5 ml) in a Teflon bottle was cooled to 0°C. Hydrofluoric acid (48-51% aqueous solution, 0.5 ml) was then added, the cooling bath was removed and the mixture was stirred at room temperature for 5 h after which time additional HF (0.2 ml) was added and the mixture was stirred for a further 2 h. Methoxytrimethylsilane (5 ml) was added and the mixture was stirred at room temperature for 10 min before being transferred to a round-bottomed flask and concentrated under reduced pressure. Further methoxytrimethylsilane (5 ml) was added to the concentrated residue, which was again concentrated to give the *phosphate (free acid)* as a colourless glass (49 mg).

The phosphate (free acid) (49 mg) was dissolved in the minimum volume of deionized water (approximately 1 ml) and cyclohexylamine (approximately 5 ml) was added. The resulting solution was stirred at room temperature for 15 min after which time toluene (5 ml) was added and the solution was concentrated. Further toluene (5 ml) was added and the mixture was again concentrated to give a pale brown solid (52 mg). ^1H, ^{13}C, ^{31}P NMR and mass spectrometry showed that the product was a mixture of the desired fully deprotected phosphate and the phosphate with one (trimethylsilyl)ethyl group still attached.

The phosphate mixture (29 mg) was then purified by ion-exchange chromatography using triethylammonium bicarbonate buffer solution to give the *(trimethylsilyl)ethyl-protected phosphate* **159** as a colourless glass (12 mg) and the desired *fully deprotected phosphate* **83b** as a colourless glass (13 mg). The 10 mM and 15 mM portions of the buffer solution eluted the trimethylsilylethyl-protected phosphate and the 20, 25, 30, 35 and 40 mM portions eluted the fully deprotected phosphate.

(1S,2S,3R)-1,2,3-Trihydroxy-4-cyclopent-4-enemethanol-5-phosphate (triethylammonium salt) **83b**; R_f 0.50 (MeCN:0.1 M NH_4Cl, 6:4); $[\alpha]_D^{23}$ +17.4 (*c* 0.58 in H_2O); $\nu_{max}(CHCl_3)/cm^{-1}$ 3311 (OH), 2980 (CH), 1650 (C=C), 1253 (P=O), 1213 (CO), 1054 (P-O-alkyl); δ_H(250 MHz; D_2O) 5.96 (1H, m, 6-H), 4.54-4.45 (4H, m, CH_2, 2-H and 3-H), 4.16 (1H, t, *J* 5.5, 1-H), 3.18 (6H, q, *J* 7.5, 3 x NCH_2), 1.25 (9H, t, *J* 7.5, 3 x NCH_2CH_3); δ_C(63 MHz; D_2O) 144.8 (1C, d, J_{PC} 7.0, C=), 129.5 (=CH), 72.6 (C-2H and C-3H), 71.8 (C-1H), 62.0 ($1CH_2$, d, J_{PC} 4.5, CH_2), 46.7 (3 x NCH_2), 8.2 (3 x NCH_2CH_3); δ_P(101 MHz; D_2O) 1.44; *m/z* (FAB) 328 (MH^+, 6%), 102 (Et_3NH, 100); [Found: MH^+, 328.15256. $C_6H_{10}O_7P.C_6H_{16}N$ requires MH, 328.15252].

(1S,2S,3R)-1,2,3-Trihydroxy-4-[(trimethylsilyl)ethyl]-phosphoryloxymethyl-cyclopent-4-ene (triethylammonium salt) **159**; R_f 0.76 (MeCN:0.1 M NH_4Cl, 6:4); $[\alpha]_D^{23}$ +8.7 (*c* 0.60 in H_2O); $\nu_{max}(CHCl_3)/cm^{-1}$ 3309 (OH), 2952 (CH), 1652 (C=C), 1249 (P=O), 1206 (CO), 1051 (P-O-alkyl), 838 ($SiMe_3$); δ_H(250 MHz; D_2O) 5.97 (1H, m, 6-H), 4.51-4.45 (4H, m, CH_2, 2-H and 3-H), 4.16 (1H, t, *J* 5.5, 1-H), 4.02-3.93 (2H, m, $POCH_2CH_2Si$), 3.17 (6H, q, *J* 7.5, 3 x NCH_2), 1.25 (9H, t, *J* 7.5, 3 x NCH_2CH_3), 1.05-0.98 (2H, m, CH_2Si), 0.00 (9H, s, $SiMe_3$); δ_C(63 MHz; D_2O) 144.5 (1C, d, J_{PC} 7.5, C=), 129.6 (=CH), 72.6 (C-2H and C-3H), 71.8 (C-1H), 64.8 ($1CH_2$, d, J_{PC} 6.0, $POCH_2CH_2Si$), 62.1 ($1CH_2$, d, J_{PC} 5.0, CH_2), 46.7 (3 x NCH_2), 18.9 ($1CH_2$, d, J_{PC} 6.0, CH_2Si), 8.2 (3 x NCH_2CH_3), -2.5 ($3CH_3$, $SiMe_3$); δ_P(101 MHz; D_2O) 1.42; *m/z* (FAB) 428 (MH^+, 3%), 102 (Et_3NH, 100), 73 ($SiMe_3$, 44); [Found: MH^+, 428.22262. $C_{11}H_{22}O_7PSi.C_6H_{16}N$ requires MH, 428.22335].

Via the sodium salt

A solution of alcohol **158** (44 mg, 0.09 mmol), acetonitrile (7.6 ml) and deionized water (1.3 ml) in a Teflon bottle was cooled to 0°C. Hydrofluoric acid (48-51% aqueous solution, 0.5 ml) was added, the cooling bath was removed and the mixture was stirred at room temperature for 6 h, after which time approximately half of the solvent was evaporated by passing nitrogen over the solution. Methoxytrimethylsilane (2 ml) was then added and the mixture was stirred at room temperature for 10 min before being transferred to a round-bottomed flask and concentrated under reduced pressure. Further methoxytrimethylsilane (2 ml) was added to the concentrated residue, which was again concentrated to give the *phosphate (free acid)* as a pale yellow glass (28 mg).

The phosphate (free acid) (28 mg) was dissolved in the minimum volume of deionized water (approximately 1 ml) and 1 M NaOH (approximately 0.15 ml) was added dropwise until a pH = 8 was obtained. The solution was then concentrated to give a pale yellow glass. Methanol:acetone (1:1) (1 ml) was added and the glass was scratched with a spatula, at which point a pale yellow solid crashed out. The solid was washed with methanol:acetone (1:1) (4 x 2 ml) before being dried under vacuum to give a pale yellow solid (18 mg). ^{1}H, ^{13}C, ^{31}P NMR and mass spectrometry showed that the product was a mixture of the fully deprotected phosphate and the phosphate with one (trimethylsilyl)ethyl group still attached.

The phosphate mixture (18 mg) was purified by ion-exchange chromatography using triethylammonium bicarbonate buffer to give the desired *fully deprotected phosphate* **83b** as a pale yellow glass (16 mg, 53%) and the *(trimethylsilyl)ethyl-protected phosphate* **159** as a colourless glass (5 mg). The 10 mM and 15 mM portions of the buffer solution eluted the trimethylsilylethyl-protected phosphate and the 20, 25, 30, 35 and 40 mM portions eluted the fully deprotected phosphate. Spectroscopic data for these compounds was consistent with that for the previous procedure.

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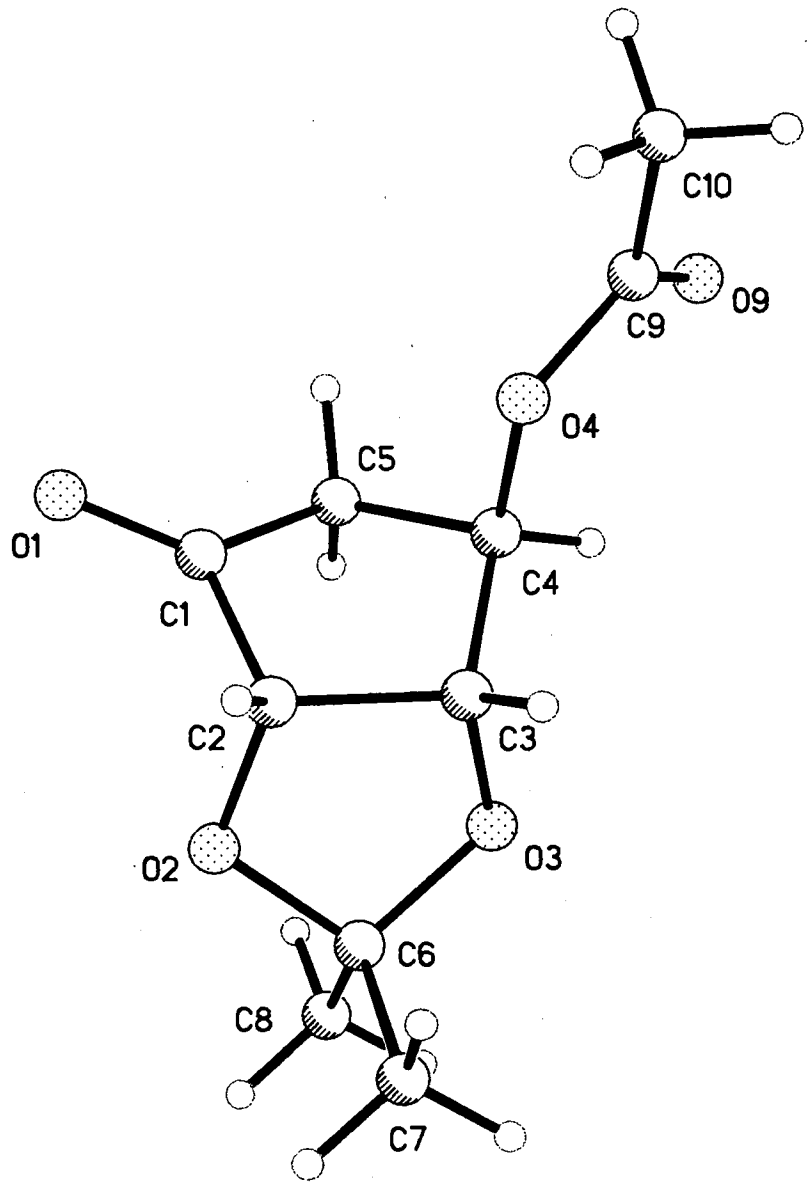
Appendices

Appendix 1

X-ray crystal structure for (2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-acetoxy-cyclopentan-1-one 118



Crystal data and structure refinement for (2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-acetoxy-cyclopentan-1-one 118

A. Crystal data

Empirical formula	$C_{10}H_{14}O_5$	
Formula weight	214.21	
Wavelength	1.54178 Å	
Temperature	220 (2) K	
Crystal system	Monoclinic	
Space group	P21	
Unit cell dimensions	a = 5.4949 (4) Å	α = 90°
	b = 6.6049 (5) Å	β = 100.049 (6)°
	c = 14.7595 (11) Å	γ = 90°
Volume	527.45 (7) $Å^3$	
Number of reflections for cell	56 ($20 < \theta < 22°$)	
Z	2	
Density (calculated)	1.349 Mg/m^3	
Absorption coefficient	0.921 mm^{-1}	
F(000)	228	

B. Data collection

Crystal description	Colourless tablet
Crystal size	0.55 x 0.43 x 0.08 mm
θ range for data collection	3.04 to 70.13°
Index ranges	$-6 \leq h \leq 6, -8 \leq k \leq 8, -17 \leq l \leq 18$
Reflections collected	2185
Independent reflections	1827 [R(int) = 0.0339]
Scan type	omega-theta
Absorption correction	Optimised numerical
	(T_{min} = 0.789, T_{max} = 0.969)

C. Solution and refinement

Solution	direct (SHELXS-97 (Sheldrick, 1990))
Refinement type	Full-matrix least-squares on F^2
Program used for refinement	SHELXL-97
Hydrogen atom placement	geometric
Hydrogen atom treatment	riding
Data/restraints/parameters	1827/1/137
Goodness-of-fit on F^2	1.044
Conventional R [$F>4\sigma$ (F)]	R1 = 0.0465 [1733 data]
Weighted R (F^2 and all data)	wR2 = 0.1261
Absolute structure parameter	-0.1 (2)
Extinction coefficient	0.014 (3)
Final maximum δ/σ	0.000
Weighting scheme	calc

$w = 1/[\backslash s^2 (Fo^2) + (0.0982P)^2 + 0.0572P]$ where $P = (Fo^2 + 2Fc^2)/3$

Largest diff. Peak and hole	0.336 and –0.303 e.$Å^{-3}$

Table 1 Atomic co-ordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for (2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-acetoxy-cyclopentan-1-one **118**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
C1	-2787 (4)	-6728 (4)	-2593 (2)	34 (1)
O1	-1255 (3)	-7881 (3)	-2792 (1)	47 (1)
C2	-3019 (3)	-4497 (4)	-2863 (1)	32 (1)
O2	-4464 (2)	-4439 (3)	-3764 (1)	38 (1)
C3	-4648 (3)	-3565 (3)	-2232 (1)	31 (1)
O3	-6991 (2)	-3281 (2)	-2823 (1)	33 (1)
C4	-4990 (4)	-5213 (4)	-1539 (2)	33 (1)
O4	-2872 (3)	-5018 (3)	-804 (1)	34 (1)
C5	-4785 (4)	-7198 (4)	-2028 (2)	37 (1)
C6	-6515 (4)	-3111 (4)	-3745 (1)	32 (1)
C7	-5869 (5)	-972 (4)	-3949 (2)	47 (1)
C8	-8706 (4)	-3933 (4)	-4396 (2)	40 (1)
C9	-3232 (4)	-5436 (3)	56 (1)	33 (1)
O9	-5191 (3)	-5986 (3)	224 (1)	43 (1)
C10	-934 (4)	-5095 (5)	741 (2)	45 (1)

Table 2 Bond lengths (Å) for (2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-acetoxy-cyclopentan-1-one **118**.

Bond	Bond Lengths (Å)
C1-O1	1.209 (3)
C1-C5	1.521 (3)
C1-C2	1.526 (3)
C2-O2	1.426 (2)
C2-C3	1.529 (3)
O2-C6	1.432 (3)
C3-O3	1.436 (2)
C3-C4	1.528 (3)
O3-C6	1.434 (2)
C4-O4	1.451 (2)
C4-C5	1.511 (3)
O4-C9	1.348 (3)
C6-C7	1.500 (4)
C6-C8	1.505 (3)
C9-O9	1.203 (3)
C9-C10	1.491 (3)

Table 3 Bond angles (°) for (2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-acetoxy-cyclopentan-1-one **118**.

Angle	Angle Size (°)
O1-C1-C5	127.2 (2)
O1-C1-C2	125.0 (2)
C5-C1-C2	107.74 (18)
O2-C2-C1	106.09 (17)
O2-C2-C3	105.48 (15)
C1-C2-C3	105.11 (17)
C2-O2-C6	108.07 (15)
O3-C3-C4	107.34 (15)
O3-C3-C2	103.94 (15)
C4-C3-C2	105.94 (18)
C6-O3-C3	107.09 (14)
O4-C4-C5	108.51 (17)
O4-C4-C3	105.13 (17)
C5-C4-C3	105.63 (18)
C9-O4-C4	117.09 (16)
C4-C5-C1	101.91 (19)
O2-C6-O3	104.20 (16)
O2-C6-C7	111.00 (19)
O3-C6-C7	110.70 (19)
O2-C6-C8	108.11 (18)
O3-C6-C8	108.65 (17)
C7-C6-C8	113.7 (2)
O9-C9-O4	122.7 (2)
O9-C9-C10	126.1 (2)
O4-C9-C10	111.22 (18)

Symmetry transformations used to generate equivalent atoms.

Table 4 Anisotropic displacement parameters (A^2 x 10^3) for (2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-acetoxy-cyclopentan-1-one **118**.

The anisotropic displacement factor exponent takes the form:

$-2\pi^2 [h^2 a^{*2} U11 + ... + 2 h k a^* b^* U12]$

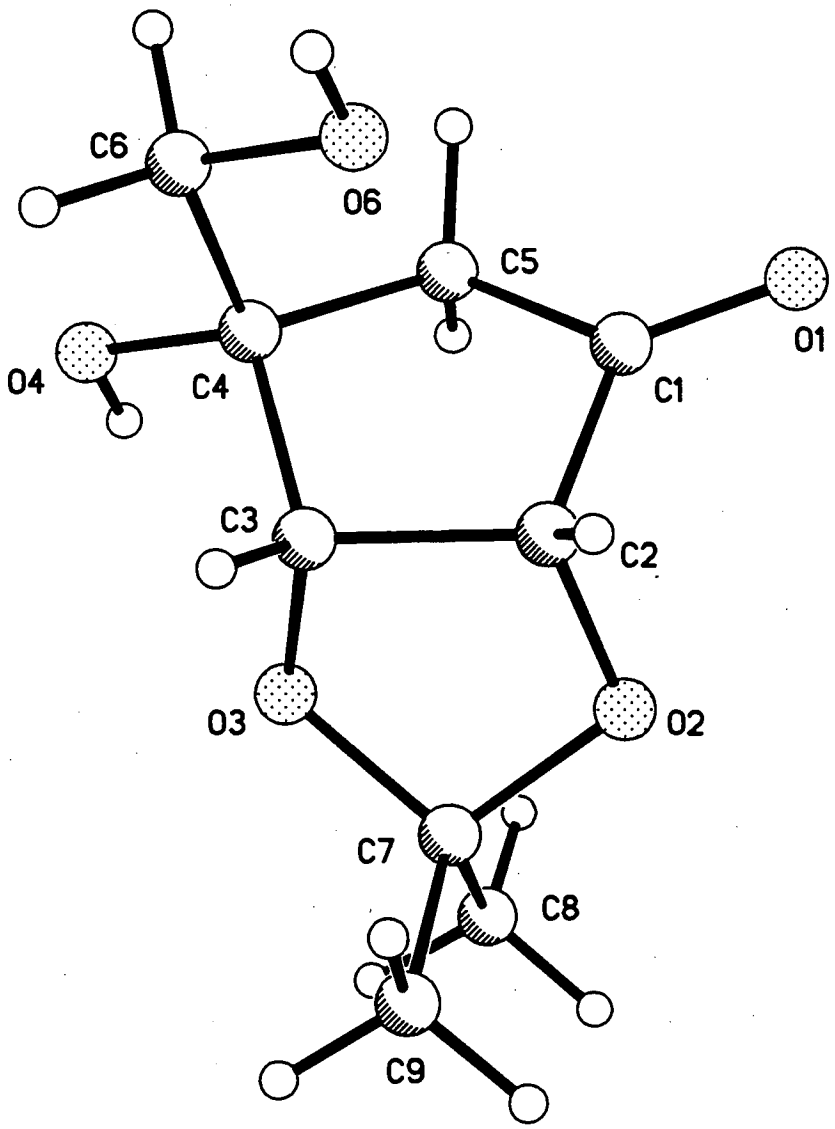
Atom	U11	U22	U33	U23	U13	U12
C1	20 (1)	47 (1)	35 (1)	-5 (1)	3 (1)	-1 (1)
O1	32 (1)	56 (1)	53 (1)	-7 (1)	7 (1)	8 (1)
C2	17 (1)	47 (1)	30 (1)	-1 (1)	4 (1)	-2 (1)
O2	21 (1)	61 (1)	31 (1)	-2 (1)	6 (1)	7 (1)
C3	16 (1)	45 (1)	31 (1)	-2 (1)	1 (1)	-1 (1)
O3	18 (1)	53 (1)	28 (1)	2 (1)	3 (1)	4 (1)
C4	17 (1)	52 (1)	30 (1)	1 (1)	2 (1)	-2 (1)
O4	19 (1)	56 (1)	28 (1)	0 (1)	3 (1)	1 (1)
C5	23 (1)	48 (1)	38 (1)	4 (1)	4 (1)	-3 (1)
C6	22 (1)	46 (1)	28 (1)	1 (1)	5 (1)	1 (1)
C7	44 (1)	53 (2)	42 (1)	6 (1)	0 (1)	-10 (1)
C8	25 (1)	59 (2)	34 (1)	-2 (1)	1 (1)	-2 (1)
C9	30 (1)	38 (1)	30 (1)	0 (1)	6 (1)	4 (1)
O9	32 (1)	60 (1)	38 (1)	3 (1)	9 (1)	-9 (1)
C10	26 (1)	73 (2)	35 (1)	-1 (1)	-2 (1)	3 (1)

Table 5 Hydrogen co-ordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for (2*S*,3*R*,4*R*)-2,3-(isopropylidenedioxy)-4-acetoxy-cyclopentan-1-one **118**.

Atom	x	y	z	U(eq)
H2	-1401	-3811	-2822	38
H3	-3959	-2295	-1936	37
H4	-6578	-5081	-1314	40
H5A	-6348	-7558	-2424	44
H5B	-4282	-8299	-1591	44
H7A	-5557	-888	-4575	71
H7B	-4401	-561	-3525	71
H7C	-7232	-86	-3880	71
H8A	-8409	-3822	-5023	60
H8B	-10170	-3162	-4333	60
H8C	-8952	-5343	-4253	60
H10A	378	-4644	426	68
H10B	-446	-6349	1065	68
H10C	-1237	-4070	1179	68

Appendix 2

X-ray crystal structure for (2*R*,3*S*,4*S*)-2,3-(isopropylidenedioxy)-4-hydroxy-methyl-4-hydroxy-cyclopentan-1-one 119



Crystal data and structure refinement for (2*R*,3*S*,4*S*)-2,3-(isopropylidenedioxy)-4-hydroxymethyl-4-hydroxy-cyclopentan-1-one 119

A. Crystal data

Empirical formula	$C_9H_{14}O_5$	
Formula weight	202.20	
Wavelength	1.54178 Å	
Temperature	220 (2) K	
Crystal system	Orthorhombic	
Space group	P212121	
Unit cell dimensions	a = 7.647 (7) Å	$\alpha = 90°$
	b = 10.586 (6) Å	$\beta = 90°$
	c = 11.853 (6) Å	$\gamma = 90°$
Volume	959.5 (11) $Å^3$	
Number of reflections for cell	77 ($20 < \theta < 22°$)	
Z	4	
Density (calculated)	1.400 Mg/m^3	
Absorption coefficient	0.975 mm^{-1}	
F(000)	432	

B. Data collection

Crystal description	Colourless tablet
Crystal size	0.43 x 0.38 x 0.23 mm
θ range for data collection	5.60 to 70.01°
Index ranges	$-9 \leq h \leq 9, -12 \leq k \leq 12, -6 \leq l \leq 14$
Reflections collected	3972
Independent reflections	1732 [R(int) = 0.0088]
Scan type	omega-theta
Absorption correction	Psi-scans
	($T_{min} = 0.331$, $T_{max} = 0.463$)

C. Solution and refinement

Solution	direct (SHELXS-97 (Sheldrick, 1990))
Refinement type	Full-matrix least-squares on F^2
Program used for refinement	SHELXL-97
Hydrogen atom placement	geometric/difmap (OH)
Hydrogen atom treatment	riding/rotating group (OH)
Data/restraints/parameters	1732/0/130
Goodness-of-fit on F^2	1.040
Conventional R [$F>4\sigma$ (F)]	R1 = 0.0325 [1716 data]
Weighted R (F^2 and all data)	wR2 = 0.0908
Absolute structure parameter	-0.1 (2)
Extinction coefficient	0.0157 (13)
Final maximum δ/σ	0.000
Weighting scheme	calc

$w = 1/[\backslash s^2 (Fo^2) + (0.0565P)^2 + 0.2747P]$ where $P = (Fo^2 + 2Fc^2)/3$

Largest diff. Peak and hole	0.240 and –0.163 e.$Å^{-3}$

Table 1 Atomic co-ordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for (2*R*,3*S*,4*S*)-2,3-(isopropylidenedioxy)-4-hydroxymethyl-4-hydroxy-cyclopentan-1-one **119**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
C1	488 (2)	4046 (2)	2433 (1)	31 (1)
O1	-405 (2)	4745 (1)	1871 (1)	47 (1)
O2	2826 (2)	3245 (1)	1325 (1)	33 (1)
C2	2478 (2)	3961 (2)	2329 (1)	29 (1)
O3	3434 (2)	1930 (1)	2775 (1)	30 (1)
C3	3062 (2)	3108 (2)	3312 (1)	27 (1)
O4	1473 (2)	1774 (1)	4668 (1)	34 (1)
C4	1452 (2)	2937 (2)	4086 (1)	27 (1)
C5	-110 (2)	3103 (2)	3291 (1)	31 (1)
O6	1526 (2)	5134 (1)	4429 (1)	41 (1)
C6	1469 (3)	3949 (2)	4995 (1)	34 (1)
C7	3941 (2)	2199 (2)	1644 (1)	31 (1)
C8	3515 (3)	1077 (2)	925 (2)	42 (1)
C9	5832 (3)	2609 (2)	1580 (2)	48 (1)

Table 2 Bond lengths (Å) for (2*R*,3*S*,4*S*)-2,3-(isopropylidenedioxy)-4-hydroxymethyl-4-hydroxy-cyclopentan-1-one **119**.

Bond	Bond Lengths (Å)
C1-O1	1.207 (2)
C1-C5	1.497 (2)
C1-C2	1.530 (3)
C2-O2	1.436 (2)
O2-C7	1.447 (2)
C2-C3	1.540 (2)
O3-C7	1.424 (2)
O3-C3	1.429 (2)
C3-C4	1.547 (2)
C4-O4	1.411 (2)
C4-C6	1.520 (2)
C4-C5	1.531 (2)
O6-C6	1.423 (2)
C7-C8	1.498 (3)
C7-C9	1.512 (3)

Table 3 Bond angles (°) for (2*R*,3*S*,4*S*)-2,3-(isopropylidenedioxy)-4-hydroxy-methyl-4-hydroxy-cyclopentan-1-one **119**.

Angle	Angle Size (°)
O1-C1-C5	127.64 (18)
O1-C1-C2	123.73 (18)
C5-C1-C2	108.59 (14)
C2-O2-C7	107.23 (12)
O2-C2-C1	106.37 (14)
O2-C2-C3	105.33 (13)
C1-C2-C3	105.23 (13)
C7-O3-C3	107.42 (12)
O3-C3-C2	103.39 (13)
O3-C3-C4	108.67 (13)
C2-C3-C4	106.66 (13)
O4-C4-C6	105.57 (13)
O4-C4-C5	114.20 (14)
C6-C4-C5	111.23 (15)
O4-C4-C3	112.56 (14)
C6-C4-C3	109.29 (14)
C5-C4-C3	104.05 (13)
C1-C5-C4	104.87 (14)
O6-C6-C4	106.68 (13)
O3-C7-O2	103.78 (13)
O3-C7-C8	108.54 (14)
O2-C7-C8	109.25 (14)
O3-C7-C9	111.43 (15)
O2-C7-C9	109.33 (16)
C8-C7-C9	114.01 (16)

Symmetry transformations used to generate equivalent atoms.

Table 4 Anisotropic displacement parameters (A^2 x 10^3) for (2*R*,3*S*,4*S*)-2,3-(isopropylidenedioxy)-4-hydroxymethyl-4-hydroxy-cyclopentan-1-one **119**.

The anisotropic displacement factor exponent takes the form:

$-2\pi^2 [h^2 a^{*2} U11 + ... + 2 h k a^* b^* U12]$

Atom	U11	U22	U33	U23	U13	U12
C1	41 (1)	28 (1)	25 (1)	-4 (1)	-3 (1)	6 (1)
O1	55 (1)	45 (1)	40 (1)	6 (1)	-7 (1)	19 (1)
O2	45 (1)	31 (1)	23 (1)	2 (1)	3 (1)	8 (1)
C2	39 (1)	23 (1)	25 (1)	1 (1)	2 (1)	-1 (1)
O3	37 (1)	26 (1)	26 (1)	2 (1)	2 (1)	3 (1)
C3	31 (1)	24 (1)	25 (1)	-1 (1)	-2 (1)	-1 (1)
O4	47 (1)	27 (1)	27 (1)	5 (1)	0 (1)	-2 (1)
C4	33 (1)	23 (1)	25 (1)	2 (1)	0 (1)	-1 (1)
C5	30 (1)	31 (1)	33 (1)	1 (1)	-1 (1)	0 (1)
O6	65 (1)	27 (1)	32 (1)	-3 (1)	0 (1)	-3 (1)
C6	47 (1)	31 (1)	25 (1)	-1 (1)	1 (1)	-2 (1)
C7	36 (1)	30 (1)	27 (1)	4 (1)	3 (1)	6 (1)
C8	57 (1)	36 (1)	34 (1)	-7 (1)	-3 (1)	11 (1)
C9	35 (1)	55 (1)	53 (1)	3 (1)	9 (1)	2 (1)

Table 5 Hydrogen co-ordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for (2*R*,3*S*,4*S*)-2,3-(isopropylidenedioxy)-4-hydroxymethyl-4-hydroxy-cyclopentan-1-one **119**.

Atom	x	y	z	U(eq)
H2	3058	4797	2332	35
H3	4086	3454	3721	32
H4	1320	1188	4214	51
H5A	-415	2300	2930	38
H5B	-1132	3418	3703	38
H6	1719	5704	4893	62
H6A	416	3890	5463	41
H6B	2496	3849	5482	41
H8A	3850	1248	151	64
H8B	2269	911	960	64
H8C	4151	346	1199	64
H9A	6138	2784	801	71
H9B	6575	1941	1869	71
H9C	5996	3366	2029	71